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(54) **POLYMORPHIC HUMAN PC-1 SEQUENCES ASSOCIATED WITH INSULIN RESISTANCE**

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(58) Field of Search 435/6; 536/23.1, 536/24.31, 24.33, 24.3

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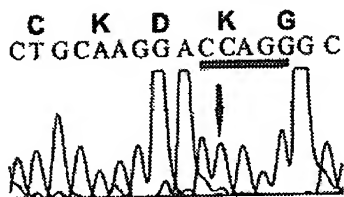
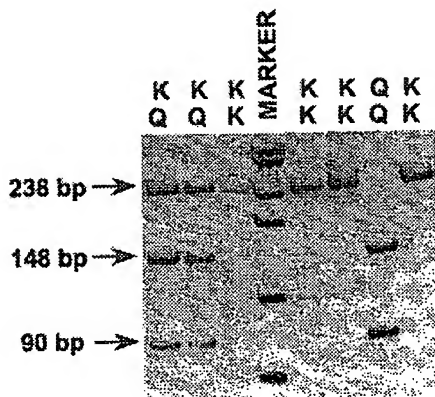
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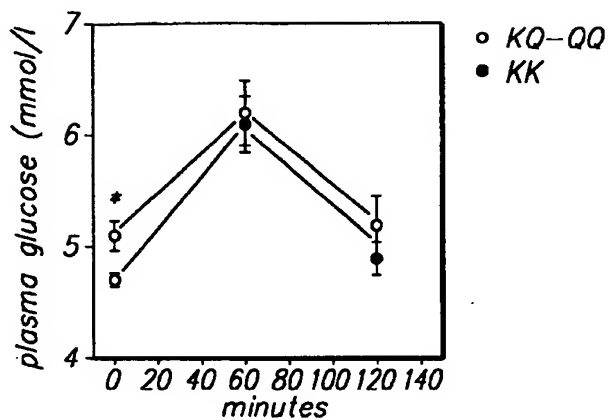
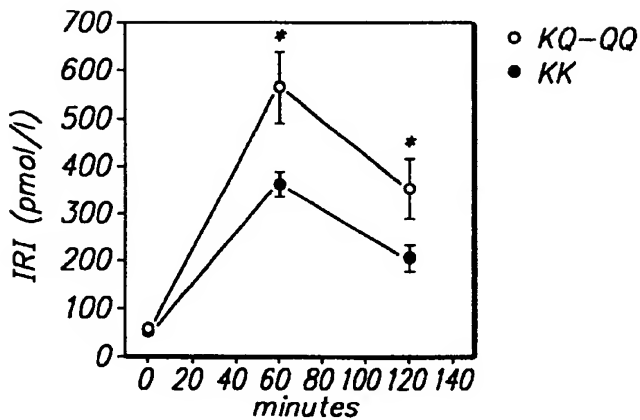
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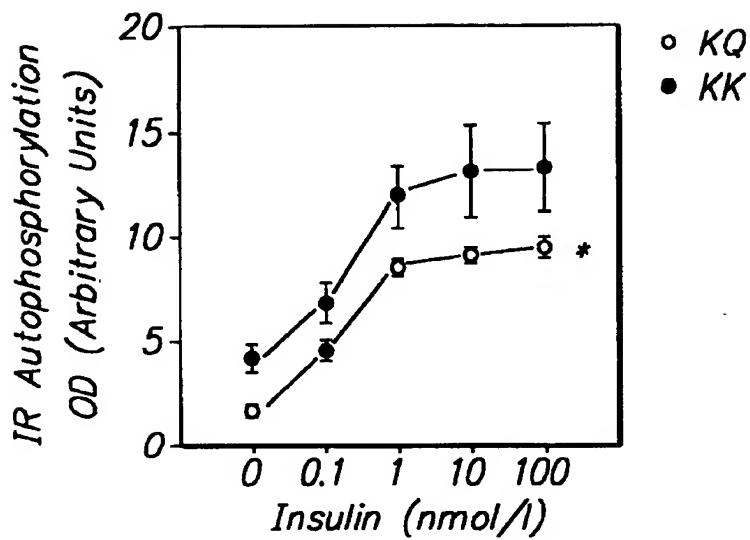
ABSTRACT

A novel polymorphism in the human PC-1 gene is characterized, which is associated with an increased predisposition to developing insulin resistance. The polymorphism affects heterozygous and homozygous carriers of the allele. The subject nucleic acids and fragments thereof, encoded polypeptides, and antibodies specific for the polymorphic amino acid sequence are useful in determining a genetic predisposition to insulin resistance. The encoded protein is useful in drug screening for compositions that affect the activity of PC-1 and insulin receptor activity or expression.

3 Claims, 3 Drawing Sheets

**FIG. 1A****FIG. 1B**

**FIG. 2A****FIG. 2B**

**FIG. 3**

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POLYMORPHIC HUMAN PC-1 SEQUENCES ASSOCIATED WITH INSULIN RESISTANCE

CROSS-REFERENCE

This application claims priority to provisional patent application No. 60/108,853, filed Nov. 18, 1998.

Insulin resistance occurs in 25% of non-diabetic, non-obese, apparently healthy individuals, and predisposes them to both diabetes and coronary artery disease. Diabetes mellitus is a major health problem in the United States affecting approximately 7% of the population. The most common form of diabetes mellitus is non-insulin-dependent diabetes mellitus (NIDDM or type II diabetes). Hyperglycemia in type II diabetes is the result of both resistance to insulin in muscle and other key insulin target tissues, and decreased beta cell insulin secretion. Longitudinal studies of individuals with a strong family history of diabetes indicate that the insulin resistance precedes the secretory abnormalities. Prior to developing diabetes these individuals compensate for their insulin resistance by secreting extra insulin. Diabetes results when the compensatory hyperinsulinemia fails. The secretory deficiency of pancreatic beta cells then plays a major role in the severity of the diabetes.

Reaven (1988) *Diabetes* 37:1595-607 were the first to have investigated insulin resistant, non-diabetic, healthy individuals from the general population who are non-obese. Strikingly, they observed that 25% of them have insulin resistance that is of a similar magnitude to that seen in type II diabetes patients. These individuals compensate by having insulin levels that are 3-4 times higher than normal. These elevated insulin levels are sufficient to maintain normoglycemia. Others have also confirmed that a large proportion of the non-diabetic population is insulin resistant. These insulin resistant, non-diabetic individuals have a much higher risk for developing type II diabetes than insulin sensitive subjects.

However, even without developing hyperglycemia and diabetes, these insulin resistant individuals pay a significant price in terms of general health. Insulin resistance results in an increased risk for having elevated plasma triglycerides (TG), lower high density lipoproteins (HDL), and high blood pressure, a cluster of abnormalities that have been termed by different investigators as either Syndrome X, the insulin resistance syndrome, or the metabolic syndrome. It is believed that either the hyperinsulinemia, insulin resistance, or both play a direct role in causing these abnormalities. Data from ethnic, family, and longitudinal studies suggest that a major component of resistance is inherited.

The cellular response to insulin is mediated through the insulin receptor (IR), which is a tetrameric protein consisting of two identical extracellular alpha subunits that bind the hormone and two identical transmembrane beta subunits that have intracellular tyrosine kinase activity. When insulin binds to the IR alpha subunit, the beta subunit tyrosine kinase domain is activated, and insulin action ensues. When insulin activates the receptor, the beta subunit is autophosphorylated at the juxtamembrane domain, the tyrosine kinase domain and the C-terminal domain. Subsequently, endogenous substrates including IRS-1, IRS-2 and SHC are tyrosine phosphorylated. These phosphorylated substrates act as docking molecules to activate SH2 domain molecules including: GRB-2 which activates the ras pathway; the p85 subunit of PI-3-kinase; protein tyrosine phosphatase PTP2/SYP; PLCγ/NCK; AKT and others.

PC-1 is a class II transmembrane glycoprotein that is located both on plasma membranes and in the endoplasmic

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reticulum. PC-1 is the same protein as liver nucleotide pyrophosphatase/alkaline phosphodiesterase 1. In addition to muscle tissue, PC-1 has been reported to be expressed in plasma and intracellular membranes of plasma cells, placenta, the distal convoluted tubule of the kidney, ducts of the salivary gland, epididymis, proximal part of the vas deferens, chondrocytes and dermal fibroblasts. PC-1 exists as a disulfide linked homodimer of 230-260 kDa; the reduced form of the protein has a molecular size of 115-135 kDa, depending on the cell type. Human PC-1 is predicted to have 873 amino acids.

PC-1 is inserted into the plasma membrane such that there is a small cytoplasmic amino terminus, and a larger extracellular carboxyl terminus. The extracellular domain of PC-1 has a high cysteine region that is involved in dimer formation, an ATP binding site and enzymatic activity which cleaves sugar-phosphate, phosphosulfate, pyrophosphate, and phosphodiesterase linkages. The active enzyme site for phosphodiesterase and pyrophosphatase contains a key threonine residue, however a mutation of this residue does not impair the ability of PC-1 to inhibit IR function.

Belli et al. (1993) *Eur J Biochem* 217(1):421-8 discloses the existence of enzymatically active water-soluble forms of PC-1. Biosynthetic studies revealed a single, monomeric, endoglycosidase-H-sensitive membrane PC-1 precursor, which was gradually converted to a disulphide-bonded, endoglycosidase-H-resistant form. The soluble form of PC-1 does not appear to arise by proteolytic cleavage from the cell surface, although cleavage inside the cell remains a possibility. The data suggest that the most likely site of cleavage is between Pro 152 and Ala 153.

PC-1 levels are increased in fibroblasts from most patients with typical NIDDM and insulin resistance. In addition, overexpression of PC-1 in transfected cultured cells reduces insulin-stimulated tyrosine kinase activity (Goldfine et al. (1998) *Mol Cell Biochem* 182:177-184). PC-1 content in fibroblasts negatively correlates with both decreased in vivo insulin sensitivity and decreased in vitro IR autophosphorylation (Frittitta et al. (1998) *Diabetes* 47:1095-1100).

In cells from insulin-resistant subjects, insulin stimulation of glycogen synthetase was decreased. PC-1 content is also elevated in fibroblasts, muscle and fat of non-diabetic insulin resistant subjects. The elevation of PC-1 content may be a primary factor in the cause of insulin resistance, although the mechanism by which PC-1 inhibits insulin receptor activity is unknown.

Many mechanisms may potentially contribute to insulin resistance. One major mechanism is the impairment of insulin receptor tyrosine kinase (IR-TK) activity, a key step in insulin receptor signalling. Several inhibitors of IR-TK have been associated to insulin resistance. Among them is PC-1, a class II transmembrane glycoprotein that is overexpressed in tissues of insulin resistant subjects. The human PC-1 gene has been assigned to the same chromosomal region (6q22-q23) where both STS D6S290 (which has been linked to type 2 diabetes in Mexican-Americans), and the gene responsible for transient neonatal diabetes map. The identification and characterization of genetic sequences involved in insulin resistance is of great medical interest.

Database References for Genetic Sequences

The human cDNA and encoded amino acid sequence for PC-1 may be accessed in Genbank, M57736 J05654. As a reference, the "K" allele is provided herein as SEQ ID NO:1, and the encoded polypeptide as SEQ ID NO:2. The "Q" allele is provided as SEQ ID NO:3, and the encoded polypeptide as SEQ ID NO:4.

SUMMARY OF THE INVENTION

Human PC-1 nucleic acids and polypeptides are provided, including promoter and intron-exon boundaries. Polymorphic sequences are provided that encode a form of the protein associated with increased insulin resistance, where a naturally occurring polymorphism of interest comprises a lys→glu substitution at position 121 of the protein, in the high cysteine region. Also provided are polymorphisms in the 3' untranslated region of PC-1. The subject nucleic acids and fragments thereof, encoded polypeptides, and antibodies specific for the polymorphic amino acid sequence are useful in determining a genetic predisposition to insulin resistance. The encoded protein is useful in drug screening for compositions that affect the activity of PC-1 and insulin receptor activity or expression. Screening methods that analyze plasma levels of soluble PC-1 are also provided, where convenient quantitation of PC-1 content is used in diagnosis of insulin resistance.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B. Sequence analysis of PC-1 exon 4. Arrows point to nucleotide N. The Q allele sequence is depicted in the upper panel, the K allele in the lower. The *Av*II restriction enzyme recognition site is underlined in the Q allele sequence. *Av*II digestion of PC-1 exon 4 amplifiers from 7 different genomic DNAs. The 238 bp amplicon is completely digested in the QQ sample, resulting two smaller 148 and 90 bp fragments. While KK samples remain undigested, KQ samples reveal a partial (50%) digestion.

FIGS. 2(A) and 2(B). Plasma glucose (A) and insulin (B) levels during an OGTT (75 g) in Q allele carriers (n=33, white circles) and KK subjects (n=68, black circles). $\bar{x} \pm p < 0.05$ vs. $\bar{x} \pm p < 0.01$ vs. KK subjects.

FIG. 3. Insulin receptor autophosphorylation in fibroblasts from Q allele carriers (n=5, white circles) and KK subjects (n=5, black circles). This function was determined by exposing cells for 10 minutes to increasing insulin concentrations (0–100 nM). Cells were then solubilized and the insulin receptor immunocaptured on plastic wells pre-coated with a monoclonal antibody specific to the insulin receptor. After washing, a biotinylated antiphosphotyrosine antibody was added followed by peroxidase-conjugated streptavidin detection assay. Data are expressed as arbitrary units, normalized for protein content. $\bar{x} \pm p < 0.01$ vs. KK subjects.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for diagnosing a predisposition to human insulin resistance. The methods comprise an analysis of germline DNA for a predisposing polymorphism in the gene encoding PC-1, where presence of the altered gene confers an increased susceptibility to insulin resistance. Human PC-1 gene and gene product compositions are provided that encode specific polymorphic forms of PC-1. Polymorphisms of interest include a coding change at position 121 of the protein, and polymorphisms of the 3' UTR.

In another embodiment of the invention, the concentration of soluble PC-1 protein in patient plasma is used as a diagnostic. PC-1 circulates in human plasma and low plasma PC-1 level is independently associated with several features of the insulin resistant "metabolic syndrome" including abdominal fat distribution, high blood pressure and, with respect to lipid metabolism, insulin resistance.

PC-1 is a class II membrane glycoprotein that inhibits activation of insulin receptor tyrosine kinase, and is associated with insulin resistance. A novel polymorphism in exon 4 of the PC-1 gene is significantly correlated with insulin resistance. The subject genes and fragments thereof, encoded protein, and antibodies specific for the insulin resistance associated forms of PC-1 are useful in characterizing patients for susceptibility to insulin resistance. Such screening methods may be used in conjunction with counseling and preventive measures.

Nucleic Acid Compositions

As used herein, the term PC-1 genes and encoded polypeptides shall be used to generally designate any of the mammalian PC-1 genes and gene products, and unless otherwise stated will be the human homolog. The sequences of the invention comprise a sequence polymorphism, generally resulting in a change in coding sequence, that confer a susceptibility to insulin resistance, and may lead to hyperglycemia and NIDDM. Such polymorphisms may be generally referred to herein as a resistance associated PC-1 sequence, or PC-1^R. Counseling and preventive measures are particularly important for such patients, and early diagnosis provides information concerning such a predisposition.

The effect of a candidate sequence polymorphism on PC-1 expression or function may be determined by kindred analysis for segregation of the sequence variation with the disease phenotype, e.g. insulin resistance, hyperglycemia, etc. A predisposing mutation will segregate with incidence of the disease. The subject mutations generally have a dominant phenotype, where a single altered allele will confer disease susceptibility. The penetrance will vary with the specific mutation.

As an alternative to kindred studies, biochemical studies are performed to determine whether a candidate sequence variation in the PC-1 coding region or control regions affects the quantity or function of the protein. The effect of a sequence variation on the interaction between PC-1 and insulin receptor or other tyrosine kinases is determined by binding studies or kinase assays, where a decreased level of inhibition or binding is indicative of a predisposing mutation. Normal PC-1 will inhibit the tyrosine kinase activity of the insulin receptor, but not other tyrosine kinases.

In one embodiment of the invention, polymorphisms of interest provide for amino acid substitutions in the extracellular domain of PC-1, particularly the cysteine-rich domain, which may substitute a charged amino acid with a neutral amino acid. In one embodiment of the invention the amino acid substitution is at a lysine residue in this region, e.g. K121 or K119. Polymorphisms at these residues, where the lysine is substituted with any of the other 19 naturally occurring amino acids, may be referred to generically as a [*121] PC-1 or [*119] PC-1 polymorphisms. Specific polymorphisms of interest substitute a neutral amino acid in place of the lysine, particularly glutamine or arginine. A naturally occurring polymorphism associated with insulin resistance comprises a lys→glu substitution at position 121 of the protein, herein referred to as "[K121Q] PC-1", or merely "[Q] PC-1".

The human [Q] PC-1 amino acid sequence is provided as SEQ ID NO:4, and the encoding gene as SEQ ID NO:3. In order to identify the subject PC-1 polymorphisms, exonic primers from the published sequence data were used to isolate genomic clones. Sequence data from the genomic clones was used to generate specific primers. These primers

were used to amplify genomic DNA. The PCR products were screened for mutations using single strand conformation polymorphism (SSCP) analysis. The specific polymorphism found in SEQ ID NO:3 was identified in a number of patients.

DNA encoding a PC-1^R protein may be cDNA or genomic DNA or a fragment thereof that encompasses the altered residue, e.g. [*121] PC-1. As known in the art, cDNA sequences have the arrangement of exons found in processed mRNA, forming a continuous open reading frame, while genomic sequences may have introns interrupting the open reading frame. The term "[*121] PC-1 gene" shall be intended to mean the open reading frame encoding such specific PC-1 polypeptides, as well as adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 1 kb beyond the coding region, in either direction. The intron-exon boundaries of the PC-1 gene are provided in the examples.

Genomic sequences of interest comprise the nucleic acids present between the initiation codon and the stop codon, including all of the introns that are normally present in a native chromosome. It may include the 3' and 5' untranslated regions found in the mature mRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb of flanking genomic DNA at either the 5' or 3' end of the coding region. The genomic DNA may be isolated as a fragment of 50 kbp or smaller; and substantially free of flanking chromosomal sequence.

The genomic PC-1 5' and 5' sequence, including specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb, but possibly more, of flanking genomic DNA at the 5' end of the transcribed region, is of particular interest. The promoter region is useful for determining the pattern of PC-1 expression, e.g. induction and inhibition of expression in various tissues, and for providing promoters that mimic these native patterns of expression. A polymorphic PC-1 regulatory sequence, i.e. including one or more of the provided 3' UTR polymorphisms, is useful for expression studies to determine the effect of sequence variations on mRNA expression and stability. The polymorphisms are also used as single nucleotide polymorphisms to detect genetic linkage to phenotypic variation in activity and expression of PC-1. The polymorphic 3' UTR sequences are provided as SEQ ID NO:6 ("A" allele); SEQ ID NO:7 ("P" allele); and SEQ ID NO:8 ("N" allele). The polymorphisms are as follows:

nucleotide position	127	136	178
SEQ ID NO: 6	G	G	C
SEQ ID NO: 7	A	C	T
SEQ ID NO: 8	A	G	T

The promoter region of PC-1 is provided as SEQ ID NO:5. The promoter region is useful for determining natural patterns of expression, particularly those that may be associated with disease. Alternatively, mutations may be introduced into the promoter region to determine the effect of altering expression in experimentally defined systems. The promoter also finds use in the construction of animal models where it is desirable to mimic the native patterns of PC-1 expression. Methods for the identification of specific DNA motifs involved in the binding of transcriptional factors are

known in the art, e.g. sequence similarity to known binding motifs, gel retardation studies, etc. For examples, see Blackwell et al. (1995) *Mol Med* 1: 194-205; Mortlock et al. (1996) *Genome Res.* 6: 327-33; and Joulin and Richard-Foy (1995) *Eur J Biochem* 232: 620-626. Specific regulatory motifs are found in the provided promoter sequence at positions: SEQ ID NO:5; 192-205; and SEQ ID NO:5, 453-458.

The nucleic acid compositions of the subject invention encode all or a part of the subject polypeptides. Fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by restriction enzyme digestion, by PCR amplification, etc. For the most part, DNA fragments will be at least about 25 nt in length, usually at least about 30 nt, more usually at least about 50 nt. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject sequence under stringent conditions, as known in the art. It is preferable to choose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other. Amplification primers of interest include the intron sequences flanking each exon, as shown in the examples, which may lie immediately outside of the coding sequence, or may span the actual junction. Use of such primers allows specific amplification of the exon sequence from genomic DNA.

The subject PC-1^R genes and associated sequences are isolated and obtained in substantial purity, generally as other than an intact mammalian chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include a PC-1 sequence or fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", i.e. flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

PC-1 Polypeptides

The subject nucleic acid compositions may be employed for producing PC-1^R protein, or fragments thereof that encompass a polymorphisms of interest, e.g. [Q121] PC-1. For expression, an expression cassette may be employed, providing for a transcriptional and translational initiation region, which may be inducible or constitutive, the coding region under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. Various transcriptional initiation regions may be employed which are functional in the expression host.

The peptide may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism or cells of a higher organism, e.g. eukaryotes such as vertebrates, particularly mammals, may be used as the expression host, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, and the like. In many situations, it may be desirable to express the subject PC-1 gene in a mammalian host, whereby the PC-1 gene product will be glycosylated, and secreted.

With the availability of the protein in large amounts by employing an expression host, the protein may be isolated

and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified protein will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to and including 100% pure. By pure is intended free of other proteins, as well as of cellular debris.

The polypeptide is used for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, and larger fragments allow for the production of antibodies over the surface of the protein. Antibodies may be raised to the normal or insulin resistant forms of PC-1. Of particular interest are antibodies that specifically recognize the insulin resistant forms of the protein, i.e. the antibodies do not bind to the normal form. Also of interest are antibodies that recognize the soluble forms of the protein. Antibodies may be raised to isolated peptides corresponding to these mutations, or to the native protein, e.g. by immunization with cells expressing PC-1, immunization with liposomes containing PC-1, etc. Such antibodies are useful in therapy and diagnosis.

Antibodies are prepared in accordance with conventional ways, where the expressed polypeptide or protein is used as an immunogen, by itself or conjugated to known immunogenic carriers, e.g. KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate. For monoclonal antibodies, after one or more booster injections, the spleen is isolated, the splenocytes immortalized, and then screened for high affinity antibody binding. The immortalized cells, e.g. hybridomas, producing the desired antibodies may then be expanded. For further description, see *Monoclonal Antibodies: A Laboratory Manual*, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1988. If desired, the mRNA encoding the heavy and light chains may be isolated and mutagenized by cloning in *E. coli*, and the heavy and light chains mixed to further enhance the affinity of the antibody. Alternatives to in vivo immunization as a method of raising antibodies include binding to phage display libraries, usually in conjunction with in vitro affinity maturation.

Phenotypic Indications

Insulin resistance is an essential feature of a great variety of clinical disorders in addition to diabetes, including coronary artery disease, hyperlipidemia, obesity and hypertension. Individuals with non-insulin dependent diabetes have insulin resistance in peripheral tissues. They have a subnormal glucose utilization in skeletal muscle, where glucose transport across the cell membrane of skeletal muscle is the rate limiting step in glucose metabolism. In adipose and muscle cells, insulin stimulates a rapid and dramatic increase in glucose uptake, primarily by promoting the redistribution of the GLUT4 glucose transporter from its intracellular storage site to the plasma membrane. Impaired glucose tolerance (IGT) is associated with a normal fasting blood glucose but an elevated postprandial blood sugar between 7.8 and 11 mmol/L (140 and 199 mg/dL). Some patients with IGT are hyperinsulinimic, and progress to NIDDM.

The response to insulin has been measured by a number of different methods, and insulin resistance has been quantified by a number of different indices. A variety of procedures have been developed to detect the presence of insulin resistance. Using any of these techniques, there is a wide

range of insulin sensitivity in normal individuals, some of whose values overlap with similar values in people with diabetes. Therefore, one cannot distinguish between nondiabetic and diabetic individuals on the basis of measures of insulin resistance.

The most widely accepted research method or 'gold standard' is the euglycemic insulin clamp technique. With this procedure, exogenous insulin is infused, so as to maintain a constant plasma insulin level above fasting, while glucose is fixed at a basal level by infusing glucose at varying rates. This glucose infusion is delivered via an indwelling catheter at a rate based on plasma glucose measurements every 5 min. When the plasma glucose level falls below basal, the glucose infusion rate is increased to return plasma glucose to basal levels and vice versa. The amount of glucose infused over time (M value) is an index of insulin action on glucose metabolism. The more glucose that has to be infused per unit time, then the more sensitive the patient is to insulin. Conversely, the insulin-resistant patient requires much less glucose to maintain basal plasma glucose levels. The effect of insulin on fuel metabolism can be assessed in the absence of the confounding effects of hypoglycemic counterregulation, endogenous insulin secretion, or variable levels of hyperglycemia, and multiple insulin actions can be assessed by using isotopes, including regulation of glucose uptake and production, inhibition of lipolysis, and changes in protein metabolism.

An alternative is the minimal model. With this procedure, glucose and insulin are sampled frequently from an indwelling catheter during an intravenous glucose tolerance test; the results are entered into a computer model, which generates a value that is an index of insulin sensitivity (called Si). The acute insulin release (AIR) in response to glucose is also determined by the test. This measure of insulin resistance correlates reasonably well with the euglycemic insulin clamp in nondiabetic subjects. Its accuracy deteriorates in diabetes because the immediate plasma insulin response to the glucose challenge is diminished. Therefore, additional maneuvers are needed to raise plasma insulin levels, such as giving tolbutamide or exogenous insulin in the course of the test.

The most practical way of assessing insulin resistance is the homeostasis model assessment (HOMAIR), involving fasting insulin and glucose levels. This value is calculated as $\text{fasting plasma insulin } (\mu\text{mol/L}) \times \text{fasting plasma glucose } (\text{mmol/L}) / 22.5$ (Matthews et al. (1985) *Diabetologia* 28:412-9). The steady-state basal plasma glucose and insulin concentrations are determined by their interaction in a feedback loop. A computer-solved model is used to predict the homeostatic concentrations which arise from varying degrees beta-cell deficiency and insulin resistance. Comparison of a patient's fasting values with the model's predictions allows a quantitative assessment of the contributions of insulin resistance and deficient beta-cell function to the fasting hyperglycaemia. The estimate of insulin resistance obtained by homeostasis model assessment correlates with estimates obtained by use of the euglycaemic clamp, the fasting insulin concentration, and the hyperglycaemic clamp. The lower limit of the top quintile of HOMA(IR) distribution (i.e. 2.77) in nonobese subjects with no metabolic disorders has been chosen as the threshold for insulin resistance in some studies (Bonora et al. (1998) *Diabetes* 47:1643-9). The results of this study documented that 1) in hypertriglyceridemia and a low LDL cholesterol state, insulin resistance is as common as in NIDDM, whereas it is less frequent in hypercholesterolemia, hyperuricemia, and hypertension; 2) the vast majority of subjects with multiple

metabolic disorders are insulin resistant; 3) in isolated hypercholesterolemia, hyperuricemia, or hypertension, insulin resistance is not more frequent than can be expected by chance alone; and 4) in the general population, insulin resistance can be found even in the absence of any major metabolic disorders.

The measurement of insulin concentration can be done in the overnight fasted condition, since in the postprandial state, glucose levels are changing rapidly and the variable levels of glucose confound the simultaneous measure of insulin levels as an index of insulin action. There is a significant correlation between fasting insulin levels and insulin action as measured by the clamp technique. Very high plasma insulin values in the setting of normal glucose levels are very likely to reflect insulin resistance. As individuals develop diabetes, plasma glucose increases and plasma insulin decreases and so the plasma insulin level no longer reflects only insulin resistance because it becomes influenced by the appearance of a β -cell defect and hyperglycemia.

Detection of PC-1 Associated Insulin Resistance

DNA from a patient having insulin resistance, as described above, suspected of association with aberrant PC-1 is analyzed for the presence of an IR polymorphism. Genetic characterization analyzes DNA or RNA, from any source, e.g. skin, cheek scrapings, blood samples, etc. The nucleic acids are screened for the presence of an insulin resistant polymorphism, e.g. SEQ ID NO:3, as compared to a normal sequence (SEQ ID NO:1, SEQ ID NO:2).

A number of methods are available for analyzing nucleic acids for the presence or absence of a specific sequence. Where large amounts of DNA are available, genomic DNA is used directly. Analysis of genomic DNA may use whole chromosomes or fractionated DNA, e.g. Southern blots, etc. Comparative Genomic Hybridization (CGH), as described in U.S. Pat. No. 5,665,549, provides methods for determining the relative number of copies of a genomic sequence. The intensity of the signals from each labeled subject nucleic acid and/or the differences in the ratios between different signals from the labeled subject nucleic acid sequences are compared to determine the relative copy numbers of the nucleic acid sequences as a function of position along the reference chromosome spread. Other methods for fluorescence in situ hybridization are known in the art, for a review, see Fox et al. (1995) *Clin Chem* 41(11):1554-1559.

Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. Cells that express PC-1 may be used as a source of mRNA, which may be assayed directly or reverse transcribed into cDNA for analysis. The nucleic acid may be amplified by conventional techniques, such as the polymerase chain reaction (PCR), to provide sufficient amounts for analysis. The use of the polymerase chain reaction is described in Saiki, et al. (1985) *Science* 239:487, and a review of techniques may be found in Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, CSH Press 1989, pp.14.2-14.33. Alternatively, various methods are known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms, for examples see Riley et al. (1990) *N.A.R.* 18:2887-2890; and Delahunty et al. (1996) *Am. J. Hum. Genet.* 58:1239-1246.

A detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red,

phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4',7'-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ^{32}P , ^{35}S , ^3H , etc. The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

The sample nucleic acid, e.g. genomic DNA, amplification product or cloned fragment, is analyzed by one of a number of methods known in the art. The nucleic acid may be sequenced by dideoxy or other methods, and the sequence of bases compared to a wild-type PC-1 sequence. Hybridization with the variant sequence may also be used to determine its presence, by Southern blots, dot blots, etc. The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilized on a solid support, as described in U.S. Pat. No. 5,445,934, or in WO95/35505, may also be used as a means of detecting the presence of variant sequences. Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence variation as alterations in electrophoretic mobility.

Alternatively, where a polymorphism creates or destroys a recognition site for a restriction endonuclease, the sample is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel or capillary electrophoresis, particularly acrylamide or agarose gels. The [Q] PC-1 allele has an A site that is not present in the [K] PC-1 allele, and this difference may be exploited for genetic screening.

Changes in the promoter or enhancer sequence that may affect expression levels of PC-1 can be compared to expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein; insertion of the variant control element into a vector with a reporter gene such as β -galactosidase, luciferase, chloramphenicol acetyltransferase, etc. that provides for convenient quantitation; and the like.

Diagnostic screening may also be performed for polymorphisms that are genetically linked to a predisposition for PC-1 associated insulin resistance, particularly through the use of microsatellite markers, e.g. the variable repeat in intron 3, or single nucleotide polymorphisms, e.g. the 3' UTR polymorphisms. Frequently the microsatellite polymorphism itself is not phenotypically expressed, but is linked to sequences that result in a disease predisposition. However, in some cases the microsatellite sequence itself may affect gene expression. Microsatellite linkage analysis may be performed alone, or in combination with direct detection of polymorphisms, as described above. The use of microsatellite markers for genotyping is well documented. For examples, see Mansfield et al. (1994) *Genomics* 24:225-233; Ziegler et al. (1992) *Genomics* 14:1026-1031; Dib et al., supra.

Microsatellite loci that are useful in the subject methods have the general formula:

$$U(R)_nU'$$

where U and U' are non-repetitive flanking sequences that uniquely identify the particular locus, R is a repeat motif, and n is the number of repeats. The repeat motif is at least 2 nucleotides in length, up to 7, usually 2-4 nucleotides in length. Repeats can be simple or complex. The flanking sequences U and U' uniquely identify the microsatellite locus within the human genome. U and U' are at least about 18 nucleotides in length, and may extend several hundred bases up to about 1 kb on either side of the repeat. Within U and U', sequences are selected for amplification primers. The exact composition of the primer sequences are not critical to the invention, but they must hybridize to the flanking sequences U and U', respectively, under stringent conditions. Criteria for selection of amplification primers are as previously discussed. To maximize the resolution of size differences at the locus, it is preferable to choose a primer sequence that is close to the repeat sequence, such that the total amplification product is between 100-500 nucleotides in length.

The number of repeats at a specific locus, n, is polymorphic in a population, thereby generating individual differences in the length of DNA that lies between the amplification primers. The number will vary from at least 1 repeat to as many as about 100 repeats or more.

The primers are used to amplify the region of genomic DNA that contains the repeats. Conveniently, a detectable label will be included in the amplification reaction, as previously described. Multiplex amplification may be performed in which several sets of primers are combined in the same reaction tube. This is particularly advantageous when limited amounts of sample DNA are available for analysis. Conveniently, each of the sets of primers is labeled with a different fluorochrome.

After amplification, the products are size fractionated. Fractionation may be performed by gel electrophoresis, particularly denaturing acrylamide or agarose gels. A convenient system uses denaturing polyacrylamide gels in combination with an automated DNA sequencer, see Hunkapillar et al. (1991) *Science* 254:59-74. The automated sequencer is particularly useful with multiplex amplification or pooled products of separate PCR reactions. Capillary electrophoresis may also be used for fractionation. A review of capillary electrophoresis may be found in Landers, et al. (1993) *BioTechniques* 14:98-111. The size of the amplification product is proportional to the number of repeats (n) that are present at the locus specified by the primers. The size will be polymorphic in the population, and is therefore an allelic marker for that locus.

Screening for polymorphisms in PC-1 may be based on the functional or antigenic characteristics of the protein. Protein truncation assays are useful in detecting deletions that may affect the biological activity of the protein. Various immunoassays designed to detect polymorphisms in PC-1 proteins may be used in screening. Where many diverse genetic mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening tools, for example by detecting the specific phosphatase activity on a PC-1 substrate. Alternatively, changes in electrophoretic mobility may be used.

Antibodies specific for an PC-1^R polymorphism may be used in staining or in immunoassays. Samples, as used herein, include cells, e.g. biopsy samples, biological fluids such as semen, blood, cerebrospinal fluid, tears, saliva,

lymph, dialysis fluid and the like; organ or tissue culture derived fluids; and fluids extracted from physiological tissues. Also included in the term are derivatives and fractions of such fluids. The cells may be dissociated, in the case of solid tissues, or tissue sections may be analyzed. Alternatively a lysate of the cells may be prepared.

Diagnosis may be performed by a number of methods to determine the absence or presence or altered amounts of normal or PC-1^R in patient cells. For example, detection may utilize staining of cells or histological sections, performed in accordance with conventional methods. Cells are permeabilized to stain cytoplasmic molecules. The antibodies of interest are added to the cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemiluminescers, or other labels for direct detection. Alternatively, a second stage antibody or reagent is used to amplify the signal. Such reagents are well known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Alternatively, the secondary antibody conjugated to a fluorescent compound, e.g. fluorescein, rhodamine, Texas red, etc. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, etc.

An alternative method for diagnosis depends on the in vitro detection of binding between antibodies and polymorphic PC-1^R in a lysate. Measuring the concentration of PC-1^R binding in a sample or fraction thereof may be accomplished by a variety of specific assays. A conventional sandwich type assay may be used. For example, a sandwich assay may first attach PC-1^R specific antibodies to an insoluble surface or support. Patient sample lysates are then added to the supports (for example, separate wells of a microtiter plate) containing antibodies. Preferably, a series of standards, containing known concentrations of normal and/or PC-1^R is assayed in parallel with the samples or aliquots thereof to serve as controls. The quantitation may then be performed by adding a labeled antibody specific for PC-1^R. Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for PC-1 as desired, conveniently using a labeling method as described for the sandwich assay.

Immunoassays may also be used in the detection of soluble PC-1 in the plasma of a patient, where quantitative and qualitative analysis may be performed. It is found that decreased levels of PC-1 in the plasma are associated with increased levels in the muscle, therefore a relatively low titer is associated with insulin resistance. In addition, the soluble PC-1 may be analyzed for the presence of a predisposing polymorphism, e.g. that Q121 protein.

A kit may be provided for practice of the subject diagnostic methods. Such a kit may contain hybridization probes that bind to a polymorphic PC-1^R sequence under hybridization conditions where the probe does not bind to a wild type PC-1 sequence. Alternatively, antibodies specific for a polymorphic PC-1^R polypeptide may be included. Such a kit will typically include positive and negative nucleic acid or polypeptide controls, and such other buffers and reagents as may be necessary to practice the method.

Modulation of Gene Expression

The PC-1 genes, gene fragments, or the encoded protein or protein fragments are useful in gene therapy to treat

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disorders associated with PC-1 insulin resistance. Expression vectors may be used to introduce a PC-1 gene into a cell. Such vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences. Transcription cassettes may be prepared comprising a transcription initiation region, the target gene or fragment thereof, and a transcriptional termination region. The transcription cassettes may be introduced into a variety of vectors, e.g. plasmid; retrovirus, e.g. lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

The gene or PC-1 protein may be introduced into tissues or host cells by any number of routes, including viral infection, microinjection, or fusion of vesicles. Jet injection may also be used for intramuscular administration, as described by Furth et al. (1992) *Anal Biochem* 205:365-368. The DNA may be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (see, for example, Tang et al. (1992) *Nature* 356:152-154), where gold microprojectiles are coated with PC-1 protein or nucleic acids encoding PC-1, then bombarded into skin cells.

Antisense molecules can be used to down-regulate expression of PC-1 in cells. The anti-sense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various mechanisms, e.g. by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner et al. (1996) *Nature Biotechnology* 14:840-844).

A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented by the antisense sequence, preferably encompassing the [Q121] PC-1 mutation. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an in vitro or animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.

Nucleic acids may be naturally occurring, e.g. DNA or RNA, or may be synthetic analogs, as known in the art. Such analogs may be preferred for use as probes because of superior stability under assay conditions. Modifications in the native structure, including alterations in the backbone, sugars or heterocyclic bases, have been shown to increase

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intracellular stability and binding affinity. Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoramidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S'-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate and 3'-NH-5'-O-phosphoramidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage.

Sugar modifications are also used to enhance stability and affinity. The α -anomer of deoxyribose may be used, where the base is inverted with respect to the natural β -anomer. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity.

Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

The antisense molecules and/or other inhibitory agents are administered by contact with cells under conditions that permit entry. The molecules may be provided in solution or in any other pharmacologically suitable form for administration, such as a liposome suspension. There are many delivery methods known in the art for enhancing the uptake of nucleic acids by cells. Useful delivery systems include Sendai virus-liposome delivery systems (see Rapaport and Shai (1994) *J. Biol. Chem.* 269:15124-15131), cationic liposomes, polymeric delivery gels or matrices, porous balloon catheters (as disclosed by Shi et al. (1994) *Circulation* 90:955-951; and Shi et al. (1994) *Gene Therapy* 1:408-414), retrovirus expression vectors, and the like.

The use of liposomes as a delivery vehicle is one method of interest. The liposomes fuse with the cells of the target site and deliver the contents of the lumen intracellularly. The liposomes are maintained in contact with the cells for sufficient time for fusion, using various means to maintain contact, such as isolation, binding agents, and the like. Liposomes may be prepared with purified proteins or peptides that mediate fusion of membranes, such as Sendai virus or influenza virus, etc. The lipids may be any useful combination of known liposome forming lipids, including cationic lipids, such as phosphatidylcholine. The remaining lipid will normally be neutral lipids, such as cholesterol, phosphatidyl serine, phosphatidyl glycerol, and the like.

The therapeutic agents are administered at a dose effective to reduce expression level of PC-1^h at least about 50%, more usually at least 80%, and preferably to substantially undetectable levels.

Genetically Modified Cells and Animals

The subject nucleic acids can be used to generate transgenic animals or site specific gene modifications in cell lines. Transgenic animals may be made through homologous recombination. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACs, and the like. The modified cells or animals are useful in the study of PC-1 function and regulation. A detectable marker, such as lac Z may be introduced into the PC-1 locus, where upregulation of PC-1 expression will result in an easily detected change in phenotype.

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DNA constructs for homologous recombination will comprise at least a portion of a polymorphic PC-1⁴ gene with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown et al. (1990) *Methods in Enzymology* 185:527-537.

For embryonic stem (ES) cells, an ES cell line may be employed, or ES cells may be obtained freshly from a host, e.g. mouse, rat, guinea pig, etc. Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected.

The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. The transgenic animals may be any non-human mammal, such as laboratory animals, domestic animals, etc. The transgenic animals may be used in functional studies, drug screening, etc., e.g. to determine the effect of a candidate drug on insulin resistance.

Drug Screening Assays

Drug screening identifies agents inhibit or otherwise modulate PC-1 function in cells. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions, transporter function, etc.

The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of PC-1. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural

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interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, enzymes, specific binding molecules, particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40° C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host for treatment of insulin resistance or hyperglycemia attributable to a defect in PC-1 function. The compounds may also be used to inhibit PC-1 function in resistance to insulin, etc. The inhibitory agents may be administered in a variety of ways, orally, topically, parenterally e.g. subcutaneously, intraperitoneally, by viral infection, intravascularly, etc. Topical treatments are of particular interest. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt. %.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories,

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capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

EXPERIMENTAL

EXAMPLE 1

Polymorphic Variant of PC-1 Associated with Insulin Resistance

Methods

Subjects

127 unrelated, healthy, non obese subjects (body mass index, BMI, $<30 \text{ Kg/m}^2$) normotensive (blood pressure $<140/90 \text{ mm Hg}$), normal glucose tolerant (by OGTT) were studied. Plasma insulin levels were measured before and during an OGTT that was carried out after 8 days on a weight-maintaining diet. Insulin stimulated glucose disposal was carried out in a subgroup of 71 subjects by the euglycaemic, hyperinsulinemic clamp.

Also studied were 132 type 2 diabetic patients (age = $66.5 \pm 8.0 \text{ yr}$, 60 male/72 female, BMI = $28.9 \pm 4.5 \text{ Kg/m}^2$) with a strong family history of diabetes (one first degree relative with type 2 diabetes at the minimum). To minimize the possible inclusion of individuals affected by late onset type 1 diabetes, patients were selected on the basis of age of diabetes onset $\geq 45 \text{ yrs}$, BMI $\geq 21 \text{ Kg/m}^2$ and no need for insulin therapy.

Informed consent was obtained from participants before entry into the study, which was approved by the local research ethics committee.

Polymorphism Screening

Overlapping cosmid clones containing the PC-1 gene were isolated by screening a human chromosome 6 specific genomic library with human full length PC-1 cDNA. Cosmids were digested with different four base cutter restriction enzymes, blotted and hybridized to oligonucleotides designed on the cDNA sequence. Positive fragments were cloned and automatically sequenced. Intron-exon junctions were deduced comparing genomic and cDNA sequences.

All exon amplimers, obtained using specific oligonucleotides as primers, were analyzed in 40 unrelated and unscreened individuals by Single Strand Conformation Polymorphism (SSCP) which was performed as follows. Amplification reaction products were denatured for 5 minutes at 87°C in 90% formamide, 20 mM EDTA, 10 mM NaOH. After denaturation samples were chilled on ice, loaded on a native 8%–12% (according to amplicon size) acrylamide (29:1 Acrylamide-Bisacrylamide) gel ($0.04 \times 20 \times 42 \text{ cm}$) in TBE and electrophoresed at 10 W constant power

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for 13–16 hours at room temperature. After the electrophoresis, gels were treated by silver staining. PCR products showing different migration patterns at SSCP were cloned in a TA-cloning vector (Stratagene) and four clones for each sample were automatically sequenced from both ends.

Exon 4 amplimers were obtained using oligonucleotides 4Fw [SEQ ID NO:9] (5'-ctgttcacttggacatgttg-3') and 4Rv [SEQ ID NO:10] (5'-gacgttggaagataaccaggttg-3') as primers. PCR products were digested using Avall restriction enzyme and run on 12% native polyacrylamide gel for 2 hours at 500V. After the electrophoresis, gels were stained by silver nitrate. On the gel K alleles are displayed as single, uncut, bands of 238 bp, while Q alleles are shown as a doublet of 148 and 90 bp.

One-hundred-sixty unscreened blood donors were genotyped as background population. All genotypings were performed in duplicate for each individual and to prevent observer bias the investigator was unaware of sample origin. Skin Fibroblast Culture and Insulin Receptor Autophosphorylation

Fibroblast cultures were established from 4-mm forearm skin-punch biopsies. ^{125}I insulin binding data were obtained by inhibition-competition studies. IR-TK (receptor autophosphorylation) was determined exposing cells for 10 min to increasing insulin (0–100 nM) concentrations. Cells were then solubilized in 50 mM Hepes buffer, pH 7.6, containing 1% Triton X-100, 1 mM PMSF, 2 mM orthovanadate and 1% BSA and IR-TK determined.

PC-1 Content in Muscle Tissue Specimens

Muscle tissue specimens were obtained from the external oblique muscle at elective abdominal surgery (cholecystectomy) and were immediately frozen in liquid nitrogen. Soluble extracts were prepared from frozen muscle tissue and PC-1 content was measured by a specific ELISA as previously described and normalized for protein content. Statistical Analysis

Group values are given as mean \pm SD. Student's t-test or Mann Whitney U test were used to compare mean values of 2 groups. One-way ANOVA and both Student-Newman-Keuls and Bonferroni t-test were used to compare mean values of more than 2 groups. Two-way ANOVA test was used to compare insulin dose-response curves of IR-TK. Chi-square test was used to compare allele frequency.

Results

The PC-1 gene has been located on chromosome 6q22–23. Analysis of a YAC contig from the region, allowed it to be more finely mapped, to between markers D6S457 and WI-3398. Only exon 4, which extends from nucleotide 447 to 571 of the cDNA and codes for an extracellular portion of PC-1, showed a polymorphic variant. When screened by SSCP analysis and sequencing it revealed a frequent first position A \rightarrow C transversion at codon 121 (considering the second in frame ATG as the start codon) (FIG. 1a). This single base change substitutes a glutamine for a lysine in a cysteine-rich region of PC-1 (SEQ ID NO:1 and SEQ ID NO:3, respectively), and creates an Avall restriction enzyme recognition site. Avall digestion of exon 4 amplimers cuts the Q allele PCR fragments, leaving the K allele undigested (FIG. 1b). In 160 uncharacterized blood donors, the Q allele frequency was 17.5%, with only 2 QQ homozygotes. The observed genotype frequencies were in agreement with those predicted by the Hardy-Weinberg equilibrium.

Having identified a PC-1 polymorphism which changes both amino acid composition and electric charge, and thus with potential biological relevance, we searched for an

association with insulin resistance. Accordingly we studied 127 unrelated, healthy, non obese, normotensive, non diabetic subjects resident in Sicily. As expected, these individuals showed a wide range of plasma insulin levels during OGTT, a finding which in the presence of normal glucose tolerance, indicates a wide range of insulin sensitivity. These data were confirmed by the euglycemic hyperinsulinemic glucose clamp, a more quantitative technique for the measurement of insulin sensitivity. In a subgroup of 71 individuals the M values for insulin stimulated glucose disposal ranged from 2.34 to 9.62 mg/Kg/min.

Table 1 summarizes the clinical features of these 2 groups. Q allele carriers showed higher fasting plasma glucose ($p<0.001$) (Table 1 and FIG. 2a) values. They also showed higher plasma insulin values at 60 ($p<0.05$) and 120 ($p<0.01$) minutes during OGTT (FIGS. 2a and 2b).

TABLE 1

Clinical Characteristics of the subject studied					
Genotype	Gender (M/F)	Age (years)	BMI (Kg/m ²)	FPG (mmol/l)	FIRI (pmol/l)
KK (n = 45)	27/18	36.6 ± 2.1	23.8 ± 0.5	4.7 ± 0.1	49.0 ± 4.0
KQ or QQ (n = 22)	18/4	40.3 ± 3.1	24.2 ± 0.8	5.1 ± 0.1*	60.0 ± 8.0

Data are expressed as mean ± SEM.

*p < 0.01 vs. KK subjects

BMI = body mass index

FPG = fasting plasma glucose

FIRI = fasting immunoreactive insulin

In the subjects studied by glucose clamp, insulin stimulated glucose disposal was lower in Q allele carriers when compared to KK allele age, sex and BMI matched subjects. No difference was observed in insulin levels at steady state during clamp studies in the 2 groups (485±165 pmol/l vs 460±78). On the average, therefore, Q allele carriers were insulin resistant and maintained normal glucose tolerance due to compensatory hyperinsulinemia. Mean blood pressure, plasma total cholesterol, HDL cholesterol and triglyceride levels were not different between the 2 groups.

Of the 2 subjects with QQ alleles, one was a 35 yr. old male who was studied by euglycemic clamp and had the second lowest M value (M=2.57 mg/Kg/min) of the all the XY males studied. The BMI (28 Kg/m²), blood pressure (138/90 mm Hg), and lipid profile (cholesterol/HDL ratio being 0.16 and triglycerides 176 mg/dl) were in the upper range of the studied individuals. The second QQ subject was a 52 yr. old female with BMI (21 Kg/m²) blood pressure, and her lipid profile was entirely normal. She did not agree to be studied by euglycemic clamp. Both QQ subjects were first degree relatives of a type 2 diabetic patient.

When subjects were subdivided into tertiles according to plasma insulin levels at 120 minutes during the OGTT (tertile 1=low, tertile 2=intermediate, and tertile=3 high insulin levels). As expected, the mean M value for glucose disposal progressively decreased from tertile 1 to tertile 3 (7.22±0.26 mg/Kg/min, n=21 vs. 5.86±0.28, n=25 and 4.89±0.23, n=25, p<0.001). Q allele frequency was similar in subjects from tertiles 1 and 2 (11.7, n=34 and 10.6%, n=33, respectively), but it was much higher in tertile 3 insulin resistant subjects (29.4%, n=34, p<0.01 when compared to the remaining 67 subjects, 11.2%). Also, in 133 type 2 diabetic patients Q allele frequency was higher (20.8%, p<0.01) than in tertile 1 and 2 subjects with no difference between obese (BMI>30 Kg/m², n=90) and non obese (n=42) patients (21.1% and 20.2%, respectively).

TABLE 2

Q allele frequency and insulin sensitivity (M value) in subjects divided in tertiles according to plasma insulin level at 120 min during OGTT (IRI 120 min)

Tertiles	1	2	3
IRI 120 min range	(1353-300 pmole/l)	(273-147 pmol/l)	(140-27 pmol/l)
Q allele frequency %	29# n = 22	15 n = 23	9 n = 22
M value (mg/kg/min)	4.50 ± 0.36*	5.46 ± 0.30**	7.21 ± 0.26 n = 17

Data are expressed as mean ± SEM. Number of subjects are given in parenthesis.

#p < 0.05 vs. tertile 3

*p < 0.01 vs. tertile 2 and 3

**p < 0.01 vs. tertile 3

In order to exclude any association of the Q allele variant with other changes in PC-1, each of the 25 exons from a QQ control were sequenced from the start to the stop codon. No other base change was detected.

In order to study IR autophosphorylation activity, cultured fibroblasts from 5 Q/K and 5 gender, age and BMI matched KK subjects were selected on the basis of a similar PC-1 protein content (50.3±8.7 and 60.8±15.4 ng/0.1 mg protein, respectively). Q/K fibroblasts showed a reduced IR autophosphorylation activity (p<0.01) (FIG. 3). Insulin binding to its receptor was studied and no difference in both total specific binding (% of bound/total radioactivity=0.52±0.10 per 0.1 mg protein and 0.55±0.11 in Q/K and KK subjects, respectively), and IC50(0.27±0.05 nmol/l and 0.26±0.08).

PC-1 content was not significantly different in muscle specimens from 8 QK and 26 KK sex, age and BMI matched subjects (36.5±5.1 ng/mg protein vs. 25.9±2.6 in QK and KK subjects, respectively).

Discussion

The data provided herein demonstrate that a PC-1 gene polymorphism (K121Q in exon 4) is associated with decreased insulin sensitivity in healthy non-diabetic individuals. Because insulin resistance is a major risk factor for the development of type 2 diabetes, Q allele carriers may be at higher risk to develop diabetes. This is supported by the high Q allele frequency observed in patients with type 2 diabetes mellitus. No association was observed with BMI both in healthy and diabetic individuals.

We previously reported that increased PC-1 content in skeletal muscle and adipose tissue is associated with insulin resistance. In addition, when cultured cells overexpress PC-1 they are insulin resistant secondary to both decreased IR tyrosine kinase activity and reduced downstream signaling steps. These latter observations indicate that an increased PC-1 content may play a role in insulin resistance through the inhibition of IR-TK activity.

PC-1 content is not significantly different in skeletal muscle from KQ with respect to KK subjects, indicating that insulin resistance in Q allele subjects is not due to an increased PC-1 protein content. Again, these data suggest that structural differences between the 2 variant proteins may account for different insulin sensitivity, independent of protein content. These data indicate PC-1 is an important candidate for the genetic regulation of whole body insulin sensitivity. PC-1 genotyping can be used for identifying individuals at risk of developing insulin resistance.

EXAMPLE 2

Fasting Plasma PC-1 and its Regulation by Insulin

A soluble form of PC-1 is generated by intracellular cleavage of its transmembrane domain, and subsequently

released by the cell. It is not known whether soluble PC-1 circulates in human plasma. The possibility of measuring PC-1 in human plasma would considerably increase the feasibility of screening studies.

A sensitive and specific ELISA was set up, and used to measure plasma PC-1 concentration before and after a 2-hour euglycemic hyperinsulinemic clamp in 22 healthy control, and 27 subjects affected by diseases known to be associated with insulin resistance (i.e. obesity and essential hypertension). The obtained results indicate that low fasting level and abnormal acute regulation by insulin of plasma PC-1 concentration are associated with several features of the "metabolic syndrome", including abdominal fat distribution, high blood pressure and low insulin sensitivity on both glucose and lipid metabolism.

Methods

Plasma PC-1 Measurement

Wells in Maxisorb plates were precoated overnight incubation at 40° C. with an affinity purified polyclonal antibody to PC-1. After washing with TBST buffer (20 mM Tris, 150 mM NaCl, 0.05% Tween-20) to remove unbound antibody, wells were blocked with 150 μ l TBST containing 1% bovine serum albumin (BSA) (30 min at 56° C.), and washed again with TBST. Then, human plasma (10–30 μ l diluted to a total volume of 100 μ l with 50 mM HEPES buffer, pH 7.6, containing 0.05% Tween-20, 1 mM PMSF, 2 mM orthovanadate, 1% BSA and 1 mg/ml bacitracin) was added to each well and PC-1 was allowed to bind overnight at 4° C. After extensive washing with TBST, a biotinylated anti-PC-1 monoclonal antibody was added in the 50 mM HEPES buffer. After 2 hr at 22° C., peroxidase-streptavidin was added and 30 min later, wells were washed again with TBST and then 100 μ l of biotinyl-tyramide solution was added. After 15 min incubation at 22° C., wells were washed with TBST and streptavidin-horseradish peroxidase was added (30 min at 22° C.). After further extensive washing, the peroxidase activity was determined calorimetrically by adding 3,3',5,5'-tetramethylbenzidine (TMB) at a concentration of 0.4 g/l in an organic base, and measuring the absorbance at 451 nm.

Muscle PC-1 Measurement

Muscle tissue specimens were obtained from the external oblique muscle at elective abdominal surgery (cholecystectomy). After adipose tissue was dissected and blood removed, specimens were immediately frozen in liquid nitrogen. Soluble extracts were prepared from frozen muscle tissue as previously described. Briefly, muscle tissue (approximately 150 mg) was pulverized under liquid nitrogen and then homogenized in 2 ml buffer (50 mM HEPES, 150 mM NaCl, 2 mM PMSF, pH 7.6) at 4° C. using a polytron homogenizer for 10 sec. at medium speed. Triton X-100 was added to a final concentration of 1%, and the homogenates solubilized for 60 min at 4° C. The material was centrifuged at 100K g for 60 min at 4° C. and the supernatants used for the PC-1 content measurement.

Statistical Analysis

One way analysis of variance (ANOVA) was utilized when means values from 3 groups were compared. Paired Student's t test was utilized to compare mean values before and after clamp.

Correlation (either "Pearson" if the data was distributed normally or "Spearman" if the data was not distributed normally) analysis was used to look for numerical relationship between values. Statistically significant correlations were confirmed by linear regression analysis. Stepwise regression analysis was utilized for multiple correlations. Data are given as mean \pm SEM.

Results

Subjects Studied

Twenty two healthy control and 27 subjects affected by either obesity (BMI>28, n=10) or essential hypertension (mean blood pressure>108 mm Hg, n=12) or both (n=5) were studied. Clinical and metabolic features of the 49 subjects are shown in Table 3. As expected, insulin sensitivity, as indicated by M values derived by euglycemic hyperinsulinemic clamp studies, was significantly reduced in obese and/or hypertensive patients as compared to normal controls.

TABLE 3

	age	sex	BMI	W/H	MBP	BG	IRI	M
Control								
mean	37	12/10	23.8	0.83	90	5.1	65	6.2
SEM	2		0.4	0.03	2	0.1	7	0.4
Insulin Resistant								
mean	47	19/8	29.2	0.92	109	5.3	80	4.8
SEM	2		0.8	0.02	3	0.1	7	0.3

Plasma PC-1 Concentration

Fasting plasma PC-1 was measured by ELISA. Human plasma produced a dilution slope that paralleled the PC-1 standard. Intra- and inter-assay coefficient of variations were <8%. Plasma PC-1 concentration ranged from 1 to 70 ng/ml with a mean \pm SE of 26.5 \pm 2.9 and a median of 24.5. No significant difference was observed between plasma PC-1 concentration in control (27.7 \pm 4.5, n=22) and insulin resistant obese and/or hypertensive (25.6 \pm 3.9, n=27) subjects.

When the 49 subjects were considered together, plasma PC-1 concentration was correlated negatively with both waist/hip ratio (-0.49 , $p=0.001$) and systolic blood pressure (-0.36 , $p=0.016$) and positively (0.40, $p=0.01$) with the ability of insulin to suppress plasma FFA (delta FFA, calculated by subtracting basal FFA from FFA after the two hour euglycemic hyperinsulinemic clamp). Plasma PC-1 concentration remained significantly correlated with the waist/hip ratio also when data were adjusted for BMI and sex ($p=0.0024$), with systolic blood pressure also when data were adjusted for sex and age ($p=0.019$) and with delta FFA also when data were adjusted for BMI, sex and waist/hip ratio ($p=0.037$).

These data demonstrate that PC-1 circulates in human plasma and that low plasma PC-1 level is independently associated with several features of the "metabolic syndrome" including abdominal fat distribution, high blood pressure and, so far as lipid metabolism is concerned, insulin resistance.

Insulin Stimulated Values

In order to verify whether insulin exerts any effect on plasma PC-1, PC-1 was measured after two hour euglycemic hyperinsulinemic clamp. Although the mean plasma PC-1 concentration in the 49 subject after clamp was not different as compared to basal plasma PC-1 level (26.3 \pm 3.9, vs. 26.5 \pm 4.1), a wide range of the individual effects of insulin infusion were observed, from subjects showing an increase to subjects showing either no change or a reduction in plasma PC-1. When subjects were divided in tertiles according to their whole body insulin sensitivity on glucose values (M values), with the most insulin sensitive in tertile 1 and the most resistant in tertile 3, insulin stimulated PC-1 concentrations were significantly higher than basal plasma PC-1 concentration in subjects from tertile 1 (21.7 \pm 5.4 vs.

Intron/Exon Structure of PC-1

The 3' flanking sequence to exon 2 (i.e. intron 3 at its 5' end) contains a GT repeat that is polymorphic, and provides a marker for genotyping of this locus. The sequences flanking the boundaries or crossing them are useful for specific amplification of the exons.

In order to verify whether plasma PC-1 concentration was related to PC-1 content in skeletal muscle, we quantified PC-1 in both plasma and biopsied external oblique muscle of 9 additional subjects. PC-1 concentration in plasma was inversely correlated with PC-1 content in muscle (-0.9 ,

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It is to be understood that this invention is not limited to the particular methodology, protocols, formulations and reagents described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a complex" includes a plurality of such complexes and reference to "the formulation" includes reference to one or more formulations and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly

understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the cell lines, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

SEQUENCE LISTING

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<221> NAME/KEY: CDS

<222> LOCATION: (164)...(2785)

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ggcgcccggg gaccgcagg cgccgcgcgt cttgctggcc cct atg gac gtg ggg	175
Met Asp Val Gly	
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Glu Glu Pro Leu Glu Lys Ala Ala Arg Thr Ala Lys Asp Pro	
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Asn Thr Tyr Lys Val Leu Ser Leu Val Leu Ser Val Cys Val Leu Thr	
25 30 35	
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Thr Ile Leu Gly Cys Ile Phe Gly Leu Lys Pro Ser Cys Ala Lys Glu	
40 45 50	
gtt aaa agt tgc aaa ggt cgc tgt ttc gag aga aca ttt ggg aac tgt	367
Val Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys	
55 60 65	
cgc tgt gat gct gcc tgt gtt gag ctt gga aac tgc tgt tta gat tac	415
Arg Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr	
70 75 80	

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gac tgc aag gac aag ggc gac tgc tgc atc aac tac agt tct gtg tgt Asp Cys Lys Asp Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys 120 125 130	559
caa ggt gag aaa agt tgg gta gaa gaa cca tgt gag agc att aat gag Gln Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu 135 140 145	607
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cct gtt att agc aaa cta aaa aaa tgt gga aca tat act aaa aac atg Pro Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met 185 190 195	751
aga ccg gta tat cca aca aaa act ttc ccc aat cac tac agc att gtc Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val 200 205 210	799
acc gga ttg tat cca gaa tct cat ggc ata atc gac aat aaa atg tat Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr 215 220 225	847
gat ccc aaa atg aat gct tcc ttt tca ctt aaa agt aaa gag aaa ttt Asp Pro Lys Met Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe 230 235 240	895
aat cct gag tgg tac aaa gga gaa cca att tgg gtc aca gct aag tat Asn Pro Glu Trp Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr 245 250 255 260	943
caa ggc ctc aag tct ggc aca ttt ttc tgg cca gga tca gat gtg gaa Gln Gly Leu Lys Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu 265 270 275	991
att aac gga att ttc cca gac atc tat aaa atg tat aat ggt tca gta Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val 280 285 290	1039
cca ttt gaa gaa agg att tta gct gtt ctt cag tgg cta cag ctt cct Pro Phe Glu Glu Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro 295 300 305	1087
aaa gat gaa aga cca cac ttt tac act ctg tat tta gaa gaa cca gat Lys Asp Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp 310 315 320	1135
tct tca ggt cat tca tat gga cca gtc agc agt gaa gtc atc aaa gcc Ser Ser Gly His Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala 325 330 335 340	1183
ttg cag agg gtt gat ggt atg gtt ggt atg ctg atg gat ggt ctg aaa Leu Gln Arg Val Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys 345 350 355	1231
gag ctg aac ttg cac aga tgc ctg aac ctc atc ctt att tca gat cat Glu Leu Asn Leu His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His 360 365 370	1279
ggc atg gaa caa ggc agt tgt aag aaa tac ata tat ctg aat aaa tat Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr 375 380 385	1327
ttg ggg gat gtt aaa aat att aaa gtt atc tat gga cct gca gct cga Leu Gly Asp Val Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg 390 395 400	1375

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ttg aga ccc tct gat gtc cca gat aaa tac tat tca ttt aac tat gaa Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu 405 410 415 420	1423
ggc att gcc cga aat ctt tct tgc cgg gaa cca aac cag cac ttc aaa Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys 425 430 435	1471
cct tac ctg aaa cat ttc tta cct aag cgt ttg cac ttt gct aag agt Pro Tyr Leu Lys His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser 440 445 450	1519
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tct gac aat gta ttt tca aat atg caa gcc ctg ttt gtt ggc tat gga Ser Asp Asn Val Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly 485 490 495 500	1663
cct gga ttc aag cat ggc att gag gct gac acc ttt gaa aac att gaa Pro Gly Phe Lys His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu 505 510 515	1711
gtc tat aac tta atg tgt gat tta ctg aat ttg aca ccg gct cct aat Val Tyr Asn Leu Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn 520 525 530	1759
aac gga act cat gga agt ctt aac cac ctt cta aag aat cct gtt tat Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr 535 540 545	1807
acg cca aag cat ccc aaa gaa gtg cac ccc ctg gta cag tgc ccc ttc Thr Pro Lys His Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe 550 555 560	1855
aca aga aac ccc aga gat aac ctt ggc tgc tca tgt aac cct tgg att Thr Arg Asn Pro Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile 565 570 575 580	1903
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gag aag att att aag cat gaa act tta ccc tat gga aga cct aga gtt Glu Lys Ile Ile Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val 600 605 610	1999
ctc cag aag gaa aac acc atc tgt ctt ctt tcc cag cac cag ttt atg Leu Gln Lys Glu Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met 615 620 625	2047
agt gga tac agc caa gac atc tta atg ccc ctt tgg aca tcc tat acc Ser Gly Tyr Ser Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr 630 635 640	2095
gtg gac aga aat gac agt ttc tct acg gaa gac ttc tcc aac tgt ctg Val Asp Arg Asn Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu 645 650 655 660	2143
tac cag gac ttt aga att cct ctt agt cct gtc cat aaa tgt tca ttt Tyr Gln Asp Phe Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe 665 670 675	2191
tat aaa aat aac acc aaa gtg agt tac ggg ttc ctg tcc cca cca caa Tyr Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln 680 685 690	2239
cta aat aaa aat tca agt gga ata tat tct gaa gct ttg ctt act aca Leu Asn Lys Asn Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr 695 700 705	2287
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710	715	720	
cat gac acc cta ctg cga aag tat gct gaa gaa aga aat ggt gtc aat His Asp Thr Leu Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn 725 730 735 740			2383
gtc gtc agt ggt cct gtg ttt gac ttt gat tat gat gga cgt tgt gat Val Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp 745 750 755			2431
tcc tta gag aat ctg agg caa aaa aga aga gtc atc cgt aac caa gaa Ser Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu 760 765 770			2479
att ttg att cca act cac ttc ttt att gtg cta aca agc tgt aaa gat Ile Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp 775 780 785			2527
aca tct cag acg cct ttg cac tgt gaa aac cta gac acc tta gct ttc Thr Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe 790 795 800			2575
att ttg cct cac agg act gat aac agc gag agc tgt gtg cat ggg aag Ile Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys 805 810 815 820			2623
cat gac tcc tca tgg gtt gaa gaa ttg tta atg tta cac aga gca cgg His Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg 825 830 835			2671
atc aca gat gtt gag cac atc act gga ctc agc ttc tat caa caa aga Ile Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg 840 845 850			2719
aaa gag cca gtt tca gac att tta aag ttg aaa aca cat ttg cca acc Lys Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr 855 860 865			2767
ttt agc caa gaa gac tga tatgtttttt atcccaaac accatgaatc Phe Ser Gln Glu Asp 870			2815
tttttgagag aaccttatat ttatatagt cctctagcta cactattgca ttgttcagaa actgtcgacc agagttagaa cggagccctc ggtgatgcgg acatctcagg gaaacttgcg tactcagcac agcagtgagg agtgttcctg ttgaatcttg cacatatattg aatgtgtaag cattgtatcac attgatcaag ttcgggggaa taaagacaga ccacacctaa aactgccttt ctgcttctct taaaggagaa gtacgtgtga acattgtctg gataccagat atttgaatct ttcttactat tggtaataaa ccttgatggc attgggcaaa cagtagactt atagtagggt tggggtagcc catgttatgt gactatcttt atgagaattt taaagtgggt ctggatatct tttaacttgg agtttcattt ctttctatgt taatcaaaaa aaaaattaac agaagccaaa atacttctga gaccttgttt caatctttgc tgtatatccc ctcaaaatoc aagttattaa tcttatgtgt tttcttttta atttttggat tggatttctt tagatttaac ggttcaaatg agttcaactt tgaggagcga tctttgaata tacttaacct ttataaaatc ttactttgta tttgtattta a			2875 2935 2995 3055 3115 3175 3235 3295 3355 3415 3475 3486
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Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly Leu Lys Pro Ser
 35 40 45
 Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr
 50 55 60
 Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys
 65 70 75 80
 Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr
 85 90 95
 Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys
 100 105 110
 Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys Cys Ile Asn Tyr
 115 120 125
 Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu
 130 135 140
 Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu Thr Pro Pro Thr
 145 150 155 160
 Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr Leu His Thr Trp
 165 170 175
 Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr
 180 185 190
 Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His
 195 200 205
 Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp
 210 215 220
 Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe Ser Leu Lys Ser
 225 230 235 240
 Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu Pro Ile Trp Val
 245 250 255
 Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe Phe Trp Pro Gly
 260 265 270
 Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys Met Tyr
 275 280 285
 Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala Val Leu Gln Trp
 290 295 300
 Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu
 305 310 315 320
 Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro Val Ser Ser Glu
 325 330 335
 Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val Gly Met Leu Met
 340 345 350
 Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu Asn Leu Ile Leu
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 Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr Ile Tyr
 370 375 380
 Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys Val Ile Tyr Gly
 385 390 395 400
 Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr Tyr Ser
 405 410 415
 Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu Pro Asn
 420 425 430
 Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro Lys Arg Leu His
 435 440 445

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Phe	Ala	Lys	Ser	Asp	Arg	Ile	Glu	Pro	Leu	Thr	Phe	Tyr	Leu	Asp	Pro
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Gln	Trp	Gln	Leu	Ala	Leu	Asn	Pro	Ser	Glu	Arg	Lys	Tyr	Cys	Gly	Ser
465						470				475				480	
Gly	Phe	His	Gly	Ser	Asp	Asn	Val	Phe	Ser	Asn	Met	Gln	Ala	Leu	Phe
			485					490						495	
Val	Gly	Tyr	Gly	Pro	Gly	Phe	Lys	His	Gly	Ile	Glu	Ala	Asp	Thr	Phe
			500					505					510		
Glu	Asn	Ile	Glu	Val	Tyr	Asn	Leu	Met	Cys	Asp	Leu	Leu	Asn	Leu	Thr
		515					520						525		
Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Lys
					530		535					540			
Asn	Pro	Val	Tyr	Thr	Pro	Lys	His	Pro	Lys	Glu	Val	His	Pro	Leu	Val
545						550				555					560
Gln	Cys	Pro	Phe	Thr	Arg	Asn	Pro	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys
					565				570					575	
Asn	Pro	Ser	Ile	Leu	Pro	Ile	Glu	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu
				580				585						590	
Thr	Val	Ala	Glu	Glu	Lys	Ile	Ile	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly
				595				600						605	
Arg	Pro	Arg	Val	Leu	Gln	Lys	Glu	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln
		610				615					620				
His	Gln	Phe	Met	Ser	Gly	Tyr	Ser	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp
625					630					635					640
Thr	Ser	Tyr	Thr	Val	Asp	Arg	Asn	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe
				645					650					655	
Ser	Asn	Cys	Leu	Tyr	Gln	Asp	Phe	Arg	Ile	Pro	Leu	Ser	Pro	Val	His
			660					665						670	
Lys	Cys	Ser	Phe	Tyr	Lys	Asn	Asn	Thr	Lys	Val	Ser	Tyr	Gly	Phe	Leu
		675				680							685		
Ser	Pro	Pro	Gln	Leu	Asn	Lys	Asn	Ser	Ser	Gly	Ile	Tyr	Ser	Glu	Ala
		690				695								700	
Leu	Leu	Thr	Thr	Asn	Ile	Val	Pro	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile
705					710					715					720
Trp	Arg	Tyr	Phe	His	Asp	Thr	Leu	Leu	Arg	Lys	Tyr	Ala	Glu	Glu	Arg
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Asn	Gly	Val	Asn	Val	Val	Ser	Gly	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp
			740					745						750	
Gly	Arg	Cys	Asp	Ser	Leu	Glu	Asn	Leu	Arg	Gln	Lys	Arg	Arg	Val	Ile
			755					760					765		
Arg	Asn	Gln	Glu	Ile	Leu	Ile	Pro	Thr	His	Phe	Phe	Ile	Val	Leu	Thr
			770				775						780		
Ser	Cys	Lys	Asp	Thr	Ser	Gln	Thr	Pro	Leu	His	Cys	Glu	Asn	Leu	Asp
785						790				795				800	
Thr	Leu	Ala	Phe	Ile	Leu	Pro	His	Arg	Thr	Asp	Asn	Ser	Glu	Ser	Cys
			805					810						815	
Val	His	Gly	Lys	His	Asp	Ser	Ser	Trp	Val	Glu	Glu	Leu	Leu	Met	Leu
			820					825						830	
His	Arg	Ala	Arg	Ile	Thr	Asp	Val	Glu	His	Ile	Thr	Gly	Leu	Ser	Phe
			835				840						845		
Tyr	Gln	Gln	Arg	Lys	Glu	Pro	Val	Ser	Asp	Ile	Leu	Lys	Leu	Lys	Thr
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865 870

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 Met Asp Val Gly
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gag gag ccg ctg gag aag gcg gcg cgc gcc cgc act gcc aag gac ccc 223
 Glu Glu Pro Leu Glu Lys Ala Ala Arg Ala Arg Thr Ala Lys Asp Pro
 5 10 15 20

aac acc tat aaa gta ctc tgc ctg gta ttg tca gta tgt gtg tta aca 271
 Asn Thr Tyr Lys Val Leu Ser Leu Val Leu Ser Val Cys Val Leu Thr
 25 30 35

aca ata ctt ggt tgt ata ttt ggg ttg aaa cca agc tgt gcc aaa gaa 319
 Thr Ile Leu Gly Cys Ile Phe Gly Leu Lys Pro Ser Cys Ala Lys Glu
 40 45 50

gtt aaa agt tgc aaa ggt cgc tgt ttc gag aga aca ttt ggg aac tgt 367
 Val Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys
 55 60 65

cgc tgt gat gct gcc tgt gtt gag ctt gga aac tgc tgt tta gat tac 415
 Arg Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr
 70 75 80

cag gag acg tgc ata gaa cca gaa cat ata tgg act tgc aac aaa ttc 463
 Gln Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe
 85 90 95 100

agg tgt ggt gag aaa agg ttg acc aga agc ctc tgt gcc tgt tca gat 511
 Arg Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp
 105 110 115

gac tgc aag gac cag gcc gac tgc tgc atc aac tac agt tct gtg tgt 559
 Asp Cys Lys Asp Gln Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys
 120 125 130

caa ggt gag aaa agt tgg gta gaa gaa cca tgt gag agc att aat gag 607
 Gln Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu
 135 140 145

cca cag tgc cca gca ggg ttt gaa acg cct cct acc ctc tta ttt tct 655
 Pro Gln Cys Pro Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser
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ttg gat gga ttc agg gca gaa tat tta cac act tgg ggt gga ctt ctt 703
 Leu Asp Gly Phe Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu
 165 170 175 180

cct gtt att agc aaa cta aaa aaa tgt gga aca tat act aaa aac atg 751
 Pro Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met
 185 190 195

aga ccg gta tat cca aca aaa act ttc ccc aat cac tac agc att gtc 799
 Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val
 200 205 210

acc gga ttg tat cca gaa tct cat gcc ata atc gac aat aaa atg tat 847
 Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr
 215 220 225

gat ccc aaa atg aat gct tcc ttt tca ctt aaa agt aaa gag aaa ttt 895
 Asp Pro Lys Met Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe

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230	235	240	
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Asn Pro Glu Trp Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr			
245	250	255	260
caa ggc ctc aag tct ggc aca ttt ttc tgg cca gga tca gat gtg gaa			991
Gln Gly Leu Lys Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu			
265	270	275	
att aac gga att ttc cca gac atc tat aaa atg tat aat ggt tca gta			1039
Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val			
280	285	290	
cca ttt gaa gaa agg att tta gct gtt ctt cag tgg cta cag ctt cct			1087
Pro Phe Glu Glu Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu			
295	300	305	
aaa gat gaa aga cca cac ttt tac act ctg tat tta gaa gaa cca gat			1135
Lys Asp Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp			
310	315	320	
tct tca ggt cat tca tat gga cca gtc agc agt gaa gtc atc aaa gcc			1183
Ser Ser Gly His Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala			
325	330	335	340
ttg cag agg gtt gat ggt atg gtt ggt atg ctg atg gat ggt ctg aaa			1231
Leu Gln Arg Val Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys			
345	350	355	
gag ctg aac ttg cac aga tgc ctg aac ctc atc ctt att tca gat cat			1279
Glu Leu Asn Leu His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His			
360	365	370	
ggc atg gaa caa ggc agt tgt aag aaa tac ata tat ctg aat aaa tat			1327
Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr			
375	380	385	
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Leu Gly Asp Val Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg			
390	395	400	
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Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu			
405	410	415	420
ggc att gcc cga aat ctt tct tgc cgg gaa cca aac cag cac ttc aaa			1471
Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys			
425	430	435	
cct tac ctg aaa cat ttc tta cct aag cgt ttg cac ttt gct aag agt			1519
Pro Tyr Leu Lys His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser			
440	445	450	
gat aga att gag ccc ttg aca ttc tat ttg gac cct cag tgg caa ctt			1567
Asp Arg Ile Glu Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu			
455	460	465	
gca ttg aat ccc tca gaa agg aaa tat tgt gga agt gga ttt cat ggc			1615
Ala Leu Asn Pro Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly			
470	475	480	
tct gac aat gta ttt tca aat atg caa gcc ctc ttt gtt ggc tat gga			1663
Ser Asp Asn Val Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly			
485	490	495	500
cct gga ttc aag cat ggc att gag gct gac acc ttt gaa aac att gaa			1711
Pro Gly Phe Lys His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu			
505	510	515	
gtc tat aac tta atg tgt gat tta ctg aat ttg aca ccg gct cct aat			1759
Val Tyr Asn Leu Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn			
520	525	530	
aac gga act cat gga agt ctt aac cac ctt cta aag aat cct gtt tat			1807
Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr			
535	540	545	
acg cca aag cat ccc aaa gaa gtg cac ccc ctg gta cag tgc ccc ttc			1855

Thr	Pro	Lys	His	Pro	Lys	Glu	Val	His	Pro	Leu	Val	Gln	Cys	Pro	Phe	
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acc	aga	aac	ccc	aga	gat	aac	ctt	ggc	tcg	tca	tgt	aac	cct	tcg	att	1903
Thr	Arg	Asn	Pro	Arg	Asp	Asn	Leu	Gly	Cys	Ser	Cys	Asn	Pro	Ser	Ile	
565					570				575						580	
tcg	ccg	att	gag	gat	ttt	caa	aca	cag	ttc	aat	ctg	act	gtg	gca	gaa	1951
Leu	Pro	Ile	Glu	Asp	Phe	Gln	Thr	Gln	Phe	Asn	Leu	Thr	Val	Ala	Glu	
				585					590					595		
gag	aag	att	att	aag	cat	gaa	act	tta	ccc	tat	gga	aga	cct	aga	gtt	1999
Glu	Lys	Ile	Ile	Lys	His	Glu	Thr	Leu	Pro	Tyr	Gly	Arg	Pro	Arg	Val	
			600					605					610			
ctc	cag	aag	gaa	aac	acc	atc	tgt	ctt	ctt	tcc	cag	cac	cag	ttt	atg	2047
Leu	Gln	Lys	Glu	Asn	Thr	Ile	Cys	Leu	Leu	Ser	Gln	His	Gln	Phe	Met	
			615				620					625				
agt	gga	tac	agc	caa	gac	atc	tta	atg	ccc	ctt	tgg	aca	tcc	tat	acc	2095
Ser	Gly	Tyr	Ser	Gln	Asp	Ile	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	
			630			635					640					
gtg	gac	aga	aat	gac	agt	ttc	tct	acg	gaa	gac	ttc	tcc	aac	tgt	ctg	2143
Val	Asp	Arg	Asn	Asp	Ser	Phe	Ser	Thr	Glu	Asp	Phe	Ser	Asn	Cys	Leu	
645				650					655					660		
tac	cag	gac	ttt	aga	att	cct	ctt	agt	cct	gtc	cat	aaa	tgt	tca	ttt	2191
Tyr	Gln	Asp	Phe	Arg	Ile	Pro	Leu	Ser	Pro	Val	His	Lys	Cys	Ser	Phe	
			665						670				675			
tat	aaa	aat	aac	acc	aaa	gtg	agt	tac	ggg	ttc	ctc	tcc	cca	cca	caa	2239
Tyr	Lys	Asn	Asn	Thr	Lys	Val	Ser	Tyr	Gly	Phe	Leu	Pro	Pro	Gln	Gln	
			680					685				690				
cta	aat	aaa	aat	tca	agt	gga	ata	tat	tct	gaa	gct	tig	ctt	act	aca	2287
Leu	Asn	Lys	Asn	Ser	Ser	Gly	Ile	Tyr	Ser	Glu	Ala	Leu	Leu	Thr	Thr	
			695				700					705				
aat	ata	gtg	cca	atg	tac	cag	agt	ttt	caa	gtt	ata	tgg	cgc	tac	ttt	2335
Asn	Ile	Val	Pro	Met	Tyr	Gln	Ser	Phe	Gln	Val	Ile	Trp	Arg	Tyr	Phe	
			710				715					720				
cat	gac	acc	cta	ctg	cga	aag	tat	gct	gaa	gaa	aga	aat	ggt	gtc	aat	2383
His	Asp	Thr	Leu	Leu	Arg	Lys	Tyr	Ala	Glu	Glu	Arg	Asn	Gly	Val	Asn	
725				730					735					740		
gtc	gtc	agt	ggt	cct	gtg	ttt	gac	ttt	gat	tat	gat	gga	cgt	tgt	gat	2431
Val	Val	Ser	Gly	Pro	Val	Phe	Asp	Phe	Asp	Tyr	Asp	Gly	Arg	Cys	Asp	
			745						750				755			
tcc	tta	gag	aat	ctg	agg	caa	aaa	aga	aga	gtc	atc	cgt	aac	caa	gaa	2479

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ttt agc caa gaa gac tga tatgttttt atccccasac accatgaatc      2815
Phe Ser Gln Glu Asp
870

tttttgagag aacottatat ttatatagt cctctagcta cactattgca ttgttcagaa 2875
actgtcgacc agagttagaa cggagccctc ggtgatgogc acatctcagg gaaacttgog 2935
tactcagcac agcagtgagg agtgttcctg ttgaattctg cacatatttg aatgtgtaag 2995
cattgtatac attgatcaag ttcgggggaa taaagacaga ccacacctaa aactgccttt 3055
ctgcttcctc taaaggagaa gtgctgtga acattgtctg gataccagat atttgaatct 3115
ttcttactat tggtaataaa ccttgatgoc attgggcaaa cagtagactt atagtagggg 3175
tggggtagcc catgttatgt gactatcttt atgagaattt taaagtgggt ctggatatct 3235
tttaacttgg agtttcattt cttttcattg taatcaaaaa aaaaattaac agaagccaaa 3295
atacttctga gaccttgttt caatctttgc tgtatatccc ctcaaaatcc aagttattaa 3355
tcttatgtgt tttcttttta attttttgat tggatttctt tagatttaat ggttcanaag 3415
agttcaactt tgaggagcga tcttggaata tacttaccta ttataaaatc ttactttgta 3475
ttgtatttta a                                     3486

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<210> SEQ ID NO 4
<211> LENGTH: 873
<212> TYPE: PRT
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 4

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Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala Arg Ala Arg Thr
 1             5             10            15

Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser Leu Val Leu Ser Val
20             25            30

Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly Leu Lys Pro Ser
35             40            45

Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys Phe Glu Arg Thr
50             55            60

Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu Leu Gly Asn Cys
65             70            75            80

Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu His Ile Trp Thr
85             90            95

Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr Arg Ser Leu Cys
100            105           110

Ala Cys Ser Asp Asp Cys Lys Asp Gln Gly Asp Cys Cys Ile Asn Tyr
115            120           125

Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu Glu Pro Cys Glu
130            135           140

Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu Thr Pro Pro Thr
145            150           155           160

Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr Leu His Thr Trp
165            170           175

Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys Cys Gly Thr Tyr
180            185           190

Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro Asn His
195            200           205

Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Ile Asp
210            215           220

Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe Ser Leu Lys Ser

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225	230	235	240
Lys Glu Lys Phe Asn Pro Glu Trp Tyr	Lys Gly Glu Pro Ile Trp Val		
	245	250	255
Thr Ala Lys Tyr Gln Gly Leu Lys	Ser Gly Thr Phe Phe Trp Pro Gly		
	260	265	270
Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys Met Tyr			
	275	280	285
Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala Val Leu Gln Trp			
	290	295	300
Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr Thr Leu Tyr Leu			
	305	310	315
Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro Val Ser Ser Glu			
	325	330	335
Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val Gly Met Leu Met			
	340	345	350
Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu Asn Leu Ile Leu			
	355	360	365
Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr Ile Tyr			
	370	375	380
Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys Val Ile Tyr Gly			
	385	390	395
Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr Tyr Ser			
	405	410	415
Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu Pro Asn			
	420	425	430
Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro Lys Arg Leu His			
	435	440	445
Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe Tyr Leu Asp Pro			
	450	455	460
Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys Tyr Cys Gly Ser			
	465	470	475
Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met Gln Ala Leu Phe			
	485	490	495
Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu Ala Asp Thr Phe			
	500	505	510
Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Asn Leu Thr			
	515	520	525
Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu Lys			
	530	535	540
Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val His Pro Leu Val			
	545	550	555
Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu Gly Cys Ser Cys			
	565	570	575
Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr Phe Asn Leu			
	580	585	590
Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr Leu Pro Tyr Gly			
	595	600	605
Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys Leu Leu Ser Gln			
	610	615	620
His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu Met Pro Leu Trp			
	625	630	635
Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser Thr Glu Asp Phe			
	645	650	655

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Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu Ser Pro Val His
 660 665 670
 Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser Tyr Gly Phe Leu
 675 680 685
 Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile Tyr Ser Glu Ala
 690 695 700
 Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser Phe Gln Val Ile
 705 710 715 720
 Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr Ala Glu Glu Arg
 725 730 735
 Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp Phe Asp Tyr Asp
 740 745 750
 Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys Arg Arg Val Ile
 755 760 765
 Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe Ile Val Leu Thr
 770 775 780
 Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys Glu Asn Leu Asp
 785 790 795 800
 Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn Ser Glu Ser Cys
 805 810 815
 Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu Leu Leu Met Leu
 820 825 830
 His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr Gly Leu Ser Phe
 835 840 845
 Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu Lys Leu Lys Thr
 850 855 860
 His Leu Pro Thr Phe Ser Gln Glu Asp
 865 870

<210> SEQ ID NO 5
 <211> LENGTH: 646
 <212> TYPE: DNA
 <213> ORGANISM: H. sapiens
 <220> FEATURE:
 <221> NAME/KEY: promoter
 <222> LOCATION: (0)...(0)
 <223> OTHER INFORMATION: PC-1 promoter sequence
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)...(646)
 <223> OTHER INFORMATION: n = A,T,C or G

<400> SEQUENCE: 5
 aaaccaact agnagctgg gaactgaat atccttaggt gtgttcagta tatgtgaacc 60
 cacgtatatt aagtgacga ttctctctc agagtaccgt aggtagtggg ggacggggcg 120
 cagaggggga gaaacagaaa gtgcgccttc tccatgttcc attgcattt ccatccagaa 180
 actcacaggt cgaccccaag actccactct ctcccgctt tgagaagccg gaccggcctc 240
 ggcggctgca tcctctctct ctcccgct ctattttggg gcccatgat ctcatgcctt 300
 ctgcagacca cacgtgcaa ttccagccca gccgcgcgcg cgaggccacg caggggcgatt 360
 cctgcaagtg tcgggagggg ggccggggcg cggggagggg acggcttggg gggaagtta 420
 agacacgccc acgtaaagga ccaaaataa ccgacacaca gagtccccga aatcagacag 480
 gaagccaaat aatccggggg gttngctgc ttgacctga ctgcagagc cggtgttagg 540
 gcggggagcc aagatctga ccgcagggg cgggcgcggc ggggagggg ggggcggggc 600

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ggcgcgcgcg gggcctatta aaggcgcggc gggcgagcg ggcgcg          646

<210> SEQ ID NO 6
<211> LENGTH: 350
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: 3'UTR
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: allele "A"

<400> SEQUENCE: 6
agccaagaag actgatatgt tttttatccc caaacacccat gaatcttttt gagagaacct      60
tatattttat atagtcctct agctacacta ttgcattggt cagaaactgt cgaccagagt      120
tagaacggag ccctcgggtg tgcggacatc tcagggaaac ttgcgtactc agcacagcag      180
tggagagtgt tcctgttgaa tcttgccatc atttgaatgt gtaagcattg tatacattga      240
tcaagttcgg ggggaataaag acagaccaca cctaaaactg cctttctgct tctcttaaaag      300
gagaagtagc tgtgaacatt gtctggatcc cagatatttg aatctttctt      350

<210> SEQ ID NO 7
<211> LENGTH: 350
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: 3'UTR
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: allele "P"

<400> SEQUENCE: 7
agccaagaag actgatatgt tttttatccc caaacacccat gaatcttttt gagagaacct      60
tatattttat atagtcctct agctacacta ttgcattggt cagaaactgt cgaccagagt      120
tagaacagag ccctcgggtg tgcggacatc tcagggaaac ttgcgtactc agcacagttag      180
tggagagtgt tcctgttgaa tcttgccatc atttgaatgt gtaagcattg tatacattga      240
tcaagttcgg ggggaataaag acagaccaca cctaaaactg cctttctgct tctcttaaaag      300
gagaagtagc tgtgaacatt gtctggatcc cagatatttg aatctttctt      350

<210> SEQ ID NO 8
<211> LENGTH: 350
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: 3'UTR
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: allele "N"

<400> SEQUENCE: 8
agccaagaag actgatatgt tttttatccc caaacacccat gaatcttttt gagagaacct      60
tatattttat atagtcctct agctacacta ttgcattggt cagaaactgt cgaccagagt      120
tagaacagag ccctcgggtg tgcggacatc tcagggaaac ttgcgtactc agcacagttag      180
tggagagtgt tcctgttgaa tcttgccatc atttgaatgt gtaagcattg tatacattga      240
tcaagttcgg ggggaataaag acagaccaca cctaaaactg cctttctgct tctcttaaaag      300
gagaagtagc tgtgaacatt gtctggatcc cagatatttg aatctttctt      350

<210> SEQ ID NO 9
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 9
ctgtgttcac ttggacatg ttg                                     23

<210> SEQ ID NO 10
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 10
gacgttgaa gataccaggt tg                                     22

<210> SEQ ID NO 11
<211> LENGTH: 109
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(109)
<223> OTHER INFORMATION: n = A,T,C or G

<400> SEQUENCE: 11
ctctcgtctg taggtccgcg gccaggcccc ggcgcccgagg agggctggga atacmgggag   60
ggcggcgccg agctcctgcg ctctcagcgc actcagcacc gggcacgga               109

<210> SEQ ID NO 12
<211> LENGTH: 279
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 12
tgagctccac cgggcccggcg gccgcctcag aactagtggg tcatgccact gtaccctagc   60
ctgggtaaca gagtaagaca ctatctctaa aaataaaaaa taagataaaa tattttttaa   120
aaaagaacc atgtaatttt ctcttttctc cctacaggta ttgagaaggt aattagggtg    180
gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtatgtgtgt gcaccgcctt attaagaatg   240
tgattgaggt aaacattatc tcctattccc aagggggtac                       279

<210> SEQ ID NO 13
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 13
agatttttgc ctacttttat taccctatct gtattttcta aagtagtatt tgaacctagt   60
gtacacctaa cttagttgta ttcggttgat ttactttga attatataat gattagaaac   120
atctgacctt tcgttcaatt ttttcagtta accaggtaag gatgagcagg gaaaaaagtg   180
gagttatggt cattaggaaa agatccacta gtcttagagc gcccgcacg cccggtggag   240
ctt                                                                    243

<210> SEQ ID NO 14
<211> LENGTH: 231
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(231)
<223> OTHER INFORMATION: n = A,T,C or G

<400> SEQUENCE: 14

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cgcggcgccc gtctagaac tagtgatca tactcacgaa gacagcaatt ctgtgttcac 60
tttggacatg ttgaattga gacataaac acattttgct gatgtttgtt tctagaacat 120
agtcaagtc aggtgctcgt tgggctctgc agcaacctgg tatcttccaa cctcttaacg 180
gggctntaca taagtgttat cttttatatt aagantcatg gctattgggc c 231

```

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<210> SEQ ID NO 15
<211> LENGTH: 313
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 15

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aatctgttca catactttgt ttgtggaac tgtcttaatg tgtctcaca gcatcacaa 60
tattattact gttaaagtgt ttcattttat ttcttgaaa atatttttagg tgagaagcag 120
ggtaaagatta tattctgagg tattaatttt ttctttttta gaagtacagc atcatttttt 180
tctttccaaa ttaagatgat aaaaataata aatcactgg tttattaaac attacagggt 240
gagtatccct tatccaaaat gtttggtag agaactgttt tggattttgg acttttttgg 300
attttgcatt att 313

```

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<210> SEQ ID NO 16
<211> LENGTH: 313
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 16

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```

cgcgagcccg ggggatcaca cagaccttag tggaaaatct tcactggacc tgtgccaaga 60
agggggtaca tcttcatttg atatgtcttg tctttgcttc tttaaacatt tttttttctt 120
tttcattacc caggtttgaa actaagttag taacttcaga gtttactgct ggaatatcac 180
catttcagtg agattgacta ggcaggcagc ctttcttgga aaagtactgg cagaacctaa 240
ctgtttcact aaacttttct aatgggcaaa gtagtgaac cttgtgtagg gcgccttacc 300
tttaataatg tga 313

```

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<210> SEQ ID NO 17
<211> LENGTH: 382
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 17

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```

taagagaaaa atgaagtcac cttaaagatt ggatttgat ccacagtggt gctttataat 60
tcactctgaa tttttatctg attaaaatcc ctctgggta atttttttta cgtgatttag 120
actgctgttg taccactgct aaatgaggta agccaattgt cagatgtatt taataacaat 180
gtttattttt ttcccttcta gaaaaatggt caccgtaagc tctgcatttc aacttctatc 240
tgtttgaaga agtgagatgg gattgtaaca ttttttgagg gaatagattt aagataaaaag 300
aaaaacaact tatcttccaa taggtagtta agtaaggaaa cccaggttct gatctttgct 360
ctgccacaaa ctactgttg ct 382

```

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<210> SEQ ID NO 18
<211> LENGTH: 312
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

```

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<400> SEQUENCE: 18

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```

actacataaa atcttaagag gttgcgtttt gccattacct gatttttttg tttttcttc 60

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cttaaaactta ttataattcc atgtagcttc agttatcgtt ttctttttga tgattttttt 120
ctgtgaatgt atttaacatt aagtaaacac aacttgcata taactctgtt tatctttttt 180
agggattaac cagtgaagtc ttgttttttc tactaaaata gtttaattatt ctcatctatt 240
tcaatcagag taaaataacc agattctcta gagcttttaa taactgattt catttagtgt 300
gtctgtggcc at 312

```

```

<210> SEQ ID NO 19
<211> LENGTH: 382
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 19

```

```

taatctctga ctatttaata tgttgttgct gcttaagagt catattacat gattattgtc 60
gtctaagtgc tgaagcttgt tgacctttaa agcattctag cactagagag gaatgcattg 120
gtgtggtatg aaacataact ttctcaagag atgaatgttg catgatttct taattttcct 180
tcattttctg ctccagattt ggaatgggta tgtgaatga attttttcta ggaatctgtaa 240
tatagaacag ctatttctta tgaattctcc tttttattga atcctgagct ttagcatttg 300
agtgatattg tggctgaaaa atgagaactg aagaactcct tctcaagag ttagataga 360
tggtaaatgg acagtaaac ta 382

```

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<210> SEQ ID NO 20
<211> LENGTH: 307
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

```

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<400> SEQUENCE: 20

```

```

gggaaataaa agttttcaaa taaaaccctt gatttcaaac acaatagatg cgaatatgca 60
ttactagct cttaatgaca ttttcaatga aaaaaactat attttacacc caaaccaattg 120
tcagccatct tttatttttg ttgttcttc attttagttc agtaagatga aaggtctgta 180
ggcaattaat ttctattgta aataactcgt ttgtagaaa tgatatacta ttttccocta 240
gactacaaca aaactttgct atttgctatg atgttttata tcgaataaaa ttcttttagta 300
aatgac 307

```

```

<210> SEQ ID NO 21
<211> LENGTH: 329
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

```

```

<400> SEQUENCE: 21

```

```

gaatttcaaa gctgtaaatt atttctcagt agaactgtta caccagtgtt ataaaattta 60
atccctatca attgaggaat tattttttcc attctgtttt tcaatgtgtt cgtaaaatat 120
tacattttga tactgtttga tttagaccac acagtgaatg agtacatttt tctcagtaat 180
tatttcatta aaccagatca tcaaaactgaa cctcgctttg aaggaggctg ctagaccatt 240
ttataagatt ctatcttttc tggaaaaagc aagtattata cacaatatta cttaatataa 300
ggatgcactt taacaaaaat aagagttgg 329

```

```

<210> SEQ ID NO 22
<211> LENGTH: 381
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

```

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<400> SEQUENCE: 22

gtcttagttt aatgtgaatc agctcattgt agttgcatcc actggcccaa atctatcaat	60
ctgtcgggtct tctcttcttt ctttgtttct tctctttttt ttttttttaa cagagatagc	120
tttatgtata aatagccatt agtggtgaag gtatcacatg aggtgtgtct tcccattctt	180
aggtcatcat catggtaatc tgaatttgca ttatttactc ttcaggataa agggctgaag	240
aaagtttact tgatggtttc ccaatttttt ctgaatgttg tagttaattc ttttttaaaa	300
atgtagtctt ttatggacag tctttaggaa aaaaatacat taataataaa atataagtga	360
aacacagaat tcacagaaac c	381

<210> SEQ ID NO 23

<211> LENGTH: 382

<212> TYPE: DNA

<213> ORGANISM: H. sapiens

<400> SEQUENCE: 23

gattttgaaa aaagtgaagt gataggtaca gctgaatttc tgtcttacct atcagatctt	60
caactaatat gagtgctaca cccatgttta acgaatttaa ccttggaagt gaaagaagtt	120
ctgctctgca tattaaattt ttgtttaaag ttacagcatg ttttgggatt tttttttctt	180
cctaggcatg gtactattca tgtaagtata tctctgtgat aactttgaat atggtcatat	240
taagaatacc ttcctttagg cggggcacag tggctcatgc ctgtaatcgc agcaacttgg	300
gaggccaaag tgggtgtgtca cctgaggtca ggagttctgag accagcctgg ccaacatggt	360
gaaccacctaa aaatacatat ac	382

<210> SEQ ID NO 24

<211> LENGTH: 361

<212> TYPE: DNA

<213> ORGANISM: H. sapiens

<400> SEQUENCE: 24

gatccaaact ctgcatttaa ataccaaggc aggtttttaa gaggttcatt aagtcattac	60
attgtagcca ctgaaggaa tttagacagac ctttagggat ctgacattct atatttttgt	120
attatgtttt aattatagta tacaatcaac tattaatctt tatgtttgtt cccctccagt	180
taactatgaa ggcattgccc gaaatctttc tgtgagatc tttattttcc attatctagt	240
tatttttact tttgtataat atatatagg agaaaagttt cagcatctat tattgggatt	300
gaaggattag aatatttttag taetctgggc caacatggaa atgctgtgta gtttaaagat	360
c	361

<210> SEQ ID NO 25

<211> LENGTH: 384

<212> TYPE: DNA

<213> ORGANISM: H. sapiens

<400> SEQUENCE: 25

ctgatgaat gtttgtgaaa aaaaatttca tatgaagtta gaagcaatt tcagaaaaag	60
ttgacacttt ttatagatat tagggaaata tctttcccta ataatatct ttcctaaaa	120
aagttgacac ttttttagat attagggaat taatagtttt tctttgtgtg ttgcaatttc	180
agtgcggggg cattgtaagt tctgacagtc tcccaggtaa acttagtctg atcggttagt	240
gattccgggt aaccattggg ccttttctaa caatattgtt atgtgaaaac tgtataagta	300
tgattctctt cactctaacc caggatttct aatgtcggcc tatggtatgt tgagtttagt	360

-continued

aattctttgt tgtggagagc tgtc 384

<210> SEQ ID NO 26
 <211> LENGTH: 381
 <212> TYPE: DNA
 <213> ORGANISM: H. sapiens
 <400> SEQUENCE: 26

aaagataga ggtgacttct taatgctttt caaagccagg tggttttatt tacogttgtg 60
 ttggtttaac aaatatagta catacttttt aatcaatgaa aataatgta tgattatcaa 120
 ttatgtttta tgaaggagct ttacatcttt aattcatata tgtcaacatt aggaatgcaa 180
 gtgagtaaac ctattatact taattggatt aaactcaag aaaaaatgat atgcaaatgt 240
 ttagacttga aaacatactg tgattatag tcttgaatga gaattaatgg aacatacttt 300
 cataaagcta ttttctcttg aacattaaag aattttgta aagttttata ttcattggct 360
 attactaaaa agtcaaaaaa c 381

<210> SEQ ID NO 27
 <211> LENGTH: 383
 <212> TYPE: DNA
 <213> ORGANISM: H. sapiens
 <400> SEQUENCE: 27

aaaactaaga gacctatcct agatgtcctt agattatgtg tgtgataggg ttaaaactat 60
 atttcccaca aagtcactg agcgtggtag ttttctctt atcttatcat aaccagtttg 120
 tatatgtaca atgtggataa cagaattttt gggaccaact tgtagacagc tgaatgcac 180
 tgataaactt ccttttctgg ccatctagcg cctgtgtggt aagtgtgaac aggtgccttt 240
 ttccctctct gaaaatagac ctgaaatagg attatcaaaa gcaggtcaca ttgtaggcaa 300
 ctttgtggag atgatggtga ggcaagacag atttttacct tcttctgac tctcagactc 360
 actgaagaaa tgtggggaac atg 383

<210> SEQ ID NO 28
 <211> LENGTH: 384
 <212> TYPE: DNA
 <213> ORGANISM: H. sapiens
 <400> SEQUENCE: 28

cataatcagta tttctattaa aaataaccta gtcttaata ctctaaaacc caagagagtt 60
 ttatactttt attttagtta aagagtaaat gactcatgta ttgggttita aaaaagttaa 120
 gatcatggca caagtctact atttgttga ttgaaacat ctaagtaact ctaccatctt 180
 gaaattatgc agatttactt cggtaagtat cgtcaagaag ttggttcag tatgtatggt 240
 ttgatagcac cctctgcata gcatgtgctg taaaaatact taataatcaa attagaattt 300
 aggaagtggg gtaggtaaac atatgtttta attctagggg ggcctgtaa atctttttgtg 360
 atatatcttt tctcttctta gt 384

<210> SEQ ID NO 29
 <211> LENGTH: 339
 <212> TYPE: DNA
 <213> ORGANISM: H. sapiens
 <400> SEQUENCE: 29

gtgaaagagc aacactcttg ccttgaaga gaaaaaaaaa tccactaata caagactatc 60

-continued

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ataaatgac tttgtctat gttggaataa tcaatctata gcggtctatg ttacaaaatt 120
taaaacatgt ctctcagctc ttacaantag ttttataacc tttttcaga ttttgccgaa 180
ggttaaggcat gctacacact caagctcggg atgtgaagca ggcattttct catcagtggt 240
aaatgcagag aactggcttg ggggtattat ttgagaataa coaatataat aaagggaggt 300
ctggaggacc acctgatgan acatagaggt ttttttgt 339

```

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<210> SEQ ID NO 30
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 30

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gtctctctaa ttgtttatgc ttgtaccctt tgtaatcagt ttttttaata gttaaaagta 60
aatcttcaat ataattaagt agaggaaagg attagatgag tgtatcacac tatatattat 120
catataatgc acactaacta catttatttt catcctgtga cccaagagaa gattagacag 180
aaatgcgaagt atttgtcacc tctttatgtg tggccatttc aaattaatga ttaagcagaa 240
cattaaatgc atagtcttct actgttcacc ttggctttat actcagttcc cgcattagag 300
gaacactgaa gaggagagta gaaaaat 327

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<210> SEQ ID NO 31
<211> LENGTH: 427
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 31

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tttaatatgt taaagcattt ttacacttta gttagaataa aagatgaata tactagtagg 60
aaaatagggg aggacatgag ctgacagcta gagcttcata attttatgat gtagttcacc 120
tttaaatatt aataaagcaa ttttctcttc tgtgcctgat atctgagagt tcttctcatt 180
ttcgtctctc aggacaccac cactgaagtt ttttctcttc ctgaccttcc ctttctctct 240
ttttgttttc tttctgtttt ataaatccta coatacatta tagggttaata tatatattac 300
ctattatata tatataaaat attacctatt ttatatatat attatatata taatatatat 360
aaagtatata tattactatt ttatatatat atagctatat atatatacct ttgtttattt 420
attgtga 427

```

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<210> SEQ ID NO 32
<211> LENGTH: 380
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

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<400> SEQUENCE: 32

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ctcatcttga aaagacttct taatatattt atttttgtaa aggaacttgac caaacacata 60
acattttccc tcgaccctgt acttgggaaa gttttacagg ttaagatgg tactcagcta 120
atttttaaaa atgctccccc aacctgaga aagtataatt tcttatgtta tttgtgaaga 180
atgaaaaagt tgtcctcttt tctctttgta gaactattca aggttaataa tgttaactct 240
atatttgata attttaatga atttgtgcac atataggcat aattcatatg tataggactt 300
atggtctaaa ttaaatgaat taataccaaa tacattctta aaggtttaac tttgagaata 360
ctagtacaca aaaattctac 380

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<210> SEQ ID NO 33
<211> LENGTH: 384

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-continued

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<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 33
ctgggtgata tagcacgact ctgtctctaa acaaaaaaca aacaaaaacg aagactgaag      60
ccaaacttga ctttatcttt atttactata aatgctaatt ttgaatcatg gtgttaattt      120
atttcacacg tcaacatggt cctctgttct ttgaaacta cactggcttc tatcttgttt      180
cagttataga ggcagtaaga acatatattca ttactcttaa aaataggaat taccatccag      240
tagaaatggg attaccatcc agttgagtcn acagaaacott ttttatccag tgcgtatgt      300
ttatgtgtat gacacttctg actacacagg aagcctcttg aaatatctga ttaattttga      360
tgtttggctc aatgttcagt aaaa                                           384

<210> SEQ ID NO 34
<211> LENGTH: 328
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 34
gttcttatat ttaattattg gttggaattt gatTTTTata tgtattaaaa gcatgctcta      60
ctgaaatatt catcaaaagg aagatagtta ttcttttctt aaatgaata ttggcatgtt      120
ttacagaaaa atgtgtggta agtagctttt gtatatttac ttgtcatgtt gaaatctag      180
acatatgcat atttgtttat gtcaccatcc tgacattaca gtgagagaaa gcacaactga      240
gtacacatgg acttcgaat tataggatgc ttttaaatTT gatcttttaa gatgacatat      300
ctttggggaa gactacccctg totgcttt                                           328

<210> SEQ ID NO 35
<211> LENGTH: 384
<212> TYPE: DNA
<213> ORGANISM: H. sapiens

<400> SEQUENCE: 35
aattaaacaa acatgcagtg tatgtattag aaggaaagct actcaagagg agagatgatg      60
cctaaacaat catgtggcac gttccacttc agagctgaaa tctcgtaaat gattaaactg      120
gggagatgga gcaacttatg aagtgaactg agtgttctct tggttaacttt tcttttatat      180
ttcttattct cctagcatgg atttaaaaaa gaaaaatatt cctatcctgc tcactggtaa      240
ttaacatagg tttaaaatgg cttcaaatgt ggccttatag acggttaaaa ttgtacctta      300
tcttggcaaa acttcagagc accagtcagt gcatgcaagg tgccattttt tattgagatg      360
cttagaatgt ttctttctgt gcac                                           384

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What is claimed is:

1. An isolated nucleic acid encoding the amino acid sequence of SEQ ID NO: 4.

2. An isolated oligonucleotide consisting of at least 18 contiguous nucleotides from an isolated nucleic acid encoding SEQ ID NO: 4, wherein said oligonucleotide includes a portion of the nucleic acid sequence that encodes a glutamine at position 121 of SEQ NO: 4.

3. A method for detecting a predisposition to insulin resistance in an individual, the method comprising:

analyzing an individual for the presence of a genetic polymorphism in the genomic sequence of a human PC-1 allele, wherein said human PC-1 allele encodes a glutamine amino acid at position 121 of SEQ ID NO: 4, and wherein the presence of said glutamine at position 121 of SEQ ID NO: 4 is indicative of a predisposition to insulin resistance.

* * * * *